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INTERVARIETAL CROSSES IN *PUCCINIA GRAMINIS*[1]

BY T. JOHNSON[2]

Abstract

Crosses made in all possible combinations between varieties *Tritici*, *Secalis*, *Avenae*, *Agrostidis*, and *Poae* of *Puccinia graminis* Pers. have shown the existence of considerable intersterility in crosses between some of the varieties. Fertility was relatively high in crosses between vars. *Tritici* and *Secalis*, *Avenae* and *Agrostidis*, *Avenae* and *Poae*, and *Agrostidis* and *Poae*. Fertility was much lower in crosses between vars. *Tritici* and *Avenae*, *Secalis* and *Avenae*, and *Secalis* and *Poae*. In crosses between certain pairs of varieties, the degree of fertility varied according to the direction of the cross. *Tritici* × *Agrostidis*, *Tritici* × *Avenae*, *Tritici* × *Poae*, *Secalis* × *Agrostidis*, and *Secalis* × *Poae* crosses succeeded much better than the reciprocal crosses. Sporidia from the F_1 teliospores of *Tritici* × *Secalis* hybrids infected barberry with the production of normal pycnia and aecia. Sporidia of *Tritici* × *Avenae* and *Secalis* × *Avenae* hybrids produced, on barberry, infections with few pycnia and these excreted little nectar; no aecia were formed but urediospores and teliospores were occasionally produced in old infections. Generally, an F_1 hybrid rust possessed a wider host range than did either of the parent varieties; but the pathogenicity of the hybrid on a given host was less than that of the parent variety that attacked that host. In hybrids of varieties that differed markedly in urediospore size, the urediospores of the F_1 hybrid rusts were intermediate in size between those of the parents. The pathogenic characteristics of the F_2 (uredial) generation were studied only in *Tritici* × *Secalis* hybrids. Variation in pathogenicity was rather restricted. The races isolated were moderately pathogenic to barley and weakly pathogenic to wheat and rye.

Introduction

Stem rust (*Puccinia graminis* Pers.) is specialized into several varieties that differ little morphologically but show striking pathogenic differences. In North America, the following five varieties are known to occur on graminaceous hosts and on barberry: *Tritici*, *Avenae*, *Secalis*, *Agrostidis*, and *Poae*. A sixth variety, *Phlei-pratensis*, also occurs on grass hosts but has not been shown to infect barberry in America though its ability to infect that host in Europe has been reported (11).

Work already published (4, 5, 8, 10) has shown that crosses may be made between the varieties *Tritici* and *Avenae*, *Tritici* and *Secalis*, and *Tritici* and *Agrostidis*. However, the work thus far reported is not extensive enough to establish the degree of interfertility of the above-mentioned varieties or to indicate conclusively what pathogenic and other properties are to be expected

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in the progeny of the crosses. The work described in the present paper was undertaken to provide further information on these matters and particularly to study crosses between varieties that hitherto had not been crossed.

Terminology

The technical terms used in connection with cereal rust studies are more or less familiar to all phytopathologists. There is, nevertheless, a possibility that such terms may be used in a somewhat different sense by different investigators. To avoid misunderstanding, definitions are given of the exact sense in which a number of these terms are used in this paper.

The word "variety" is used to signify the *forma specialis* of Eriksson.

The word "pustule" is used with reference to all spore forms of the rust except the sporidia (basidiospores). In connection with the spore forms on barberry, it refers to infections bearing a single group of pycnia (pycnial pustules) or aecia (aecial pustules). In the spore forms on grass hosts, it refers to a single sorus of urediospores or teliospores.

"Cross" refers to the transfer of the exudate or nectar from the pycnia (which contains the pycniospores) of one or more pycnial pustules of one variety of the rust to a single haploid pustule of another variety. In the sense used, a cross does not necessarily imply the production of aeciospores. A dozen crosses may be made and possibly only one or two may succeed in producing aecia. In crossing any two varieties of the rust, the crosses are made in opposite directions according to which variety is used as the "female", i.e., nectar-receiving parent. A cross reported as *Agrostidis* × *Avenae* signifies that a haploid *Agrostidis* pustule received an application of *Avenae* pycnial nectar. *Avenae* × *Agrostidis* indicates the reverse procedure. Combinations without reference to the direction in which crosses were made are occasionally referred to as *Tritici–Agrostidis*, *Avenae–Secalis*, etc.

The term "hybrid" is used to signify the dikaryon formed when, as a result of a cross, a haploid nucleus of one rust variety comes into association with a haploid nucleus of another variety. This practice is open to objection by those who maintain that the hybrid does not come into being until the fusion of these two nuclei in the teliospore. A distinction could be made by regarding the dikaryon as an "associant" or a "pseudohydrid" or a "prohybrid"; but in the present paper the attitude is taken that such a distinction is unnecessary as it is known that the rust dikaryon does display the essentials of hybridity, such as dominance, recessiveness, and intermediacy. In other words, the genes in the dikaryon act as if they were present in a diploid nucleus.

Materials and Methods

In the varieties *Tritici* and *Avenae*, known physiologic races of ascertained purity were used in the crosses, the teliospores being produced on wheat or oat plants grown in the greenhouse. In the varieties *Secalis*, *Agrostidis*,

and *Poae*, the teliospores employed were collected in nature on grass hosts congenial to these three varieties. Data on the actual collections used are given below:

Variety	Host	Date of collection	Locality
Secalis	*Agropyron repens*	May, 1942	McKellar, Ont.
"	" "	May 13, 1944	" "
"	" "	May 23, 1945	Fredericton, N.B.
Agrostidis	*Agrostis alba*	Sept. 10, 1943	Ottawa, Ont.
"	Same source, greenhouse-formed telia		
"	*Agrostis* sp.	May 23, 1945	Fredericton, N.B.
"	" *tenuis, A. Reuteri, A. alba*	April, 1946	" "
Poae	*Poa compressa*	May 13, 1944	Ottawa, Ont.
"	Same source, greenhouse-formed telia		
"	*Berberis vulgaris*	1945	Guelph, Ont.
	Greenhouse-formed telia on *Poa compressa*		

In the collections of telia made in nature, precautions were taken to ensure, as far as possible, that the correct variety was used in each case. As each collection was made or received, telia-bearing straws with heads or panicles attached were separated out, and so long as they were available only such straws were used for the infection of barberry. Later, it became necessary, in certain of the collections, to use straws with no heads attached. But whether or not heads were attached, the aeciospores that developed were tested for their infectivity on wheat, oats, rye, barley, and the required grass hosts to secure evidence that the correct variety of the rust was being used.

As a sparse distribution of pycnial pustules is desirable for crossing purposes, attempts were made to inoculate barberry plants in such a way that the infections were widely distributed over the leaves and separated from each other by a distance of at least one centimeter. Infection of barberry was accomplished by suspending the telia-bearing straws over a plant enclosed in a lantern chimney, the wet straws being pressed firmly against a blotting paper in a Petri plate inverted over the top of the chimney. The length of time the telia were suspended over the plants varied from a few hours to 24 hr. or more, depending on the rate of sporidial discharge as determined prior to inoculation.

After inoculation, the infected plants were kept in close-mesh screen cages for at least three weeks, or until development of aecia was general. It was then assumed that isolated pustules that had not produced aecia were haploid, and these were therefore selected for use in the crossing studies. Each of the selected pustules on a plant was given a number (inscribed in India ink on the adjacent leaf area), and thereafter diagrams showing the location of each pustule were entered in the record book.

The application of pycnial nectar of one variety of the rust to pustules of another variety was then carried out by means of a platinum wire loop, which was sterilized between successive transfers by passing through a flame. In most crosses, nectar was transferred from many pustules of one variety of the rust to each individual pustule of another variety so as to favor as far as possible the formation of aecia in each nectar-receiving pustule. Following the transfer of nectar, the plants were replaced in the screen cages and the nectar-receiving pustules were examined every two or three days to record the development or nondevelopment of aecia.

When the aecia of any given pustule had reached appropriate development, they were removed by means of a small, sterile, metal spatula and placed in a drop of water on a sterile glass slide. There they were crushed to liberate the aeciospores and the resulting spore suspension was immediately applied to seedling leaves of the appropriate cereals—wheat and oats in the *Tritici–Avenae* crosses, wheat and rye in the *Tritici–Secalis* crosses, etc. Cultures thus established were propagated on the most suitable host and studied for their pathogenicity toward cereal varieties and grasses.

Fertility Between Varieties of the Rust

It was shown by Craigie (1, 2, 3) that haploid pustules of *Puccinia graminis*, as also of *P. Helianthi* Schw., fell into two sex or interfertility groups, which he designated as (+) and (−). Application of pycniospore-bearing nectar of a (+) pustule to a (−) pustule, or vice versa, resulted in the production of aecia in the pustule receiving the application, providing both pustules belonged to the same variety of *P. graminis*.

In crosses between two physiologic races of the same variety of *P. graminis*, there appears to be little if any intersterility. When nectar is transferred from a haploid pustule of one physiologic race to several haploid pustules of another, the usual result is that about half of the nectar-receiving pustules produce aecia. If composite nectar derived from many pustules of one race, and therefore containing both (+) and (−) pycniospores, is applied to a number of pustules of another race, aecia are usually formed in all pustules receiving the application. In either case it may be said that fertility between the two races is complete. The results of numerous intravarietal crosses in the *Tritici* variety and in the *Avenae* variety indicate that within these varieties fertility between the different physiologic races is of a relatively high order. Crosses between different *Tritici* races, involving 164 haploid pustules, indicated 72% interfertility, and crosses between *Avenae* races, in which 137 pustules were employed, showed 100% interfertility.

In crosses between varieties of stem rust (intervarietal crosses), there is, on the other hand, evidence of a considerable degree of intersterility except possibly in *Tritici–Secalis* and *Agrostidis–Poae* crosses. Stakman, Levine, and Cotter (10) obtained no aecia as a result of the application of *Tritici* nectar to haploid *Agrostidis* pustules but obtained aecia in all *Tritici* pustules

that received *Agrostidis* nectar. Johnson, Newton, and Brown (4) applied nectar of haploid *Tritici* pustules to 36 haploid *Agrostidis* pustules with the result that only three produced aecia, which in all three cases were attributed to fortuitous selfing of the *Agrostidis* variety. Aecia of hybrid origin were, however, obtained in one of the 14 *Tritici* pustules receiving applications of *Agrostidis* nectar. With regard to *Tritici–Agrostidis* crosses, the published evidence indicates a considerable degree of intersterility at least on the *Agrostidis* side, i.e., when haploid *Agrostidis* pustules receive *Tritici* nectar. Johnson and Newton (5) also furnished some evidence of intersterility in *Tritici–Avenae* crosses. Aecia were formed in only one of 13 haploid *Avenae* pustules receiving a mass transfer of nectar derived from many *Tritici* pustules, and similarly aecia were formed in only one of 19 *Tritici* pustules that received *Avenae* nectar.

To the above-mentioned data may now be added similar information derived from the crosses under consideration in the present paper. In Table I a summary is given of all intervarietal crosses made by the writer, and, in addition, the *Tritici–Secalis*, *Tritici–Avenae*, and *Tritici–Agrostidis* crosses mentioned above (4, 5). In the great majority of the crosses, a mass transfer of nectar was made from many pustules of one variety of the rust to individual haploid pustules of another variety, i.e., (+) and (−) → (+) or (−) mating types. In a number of the earlier crosses, however, nectar transfers were made from a single haploid pustule of one variety to several haploid pustules of another, i.e., (+) or (−) → (+) or (−) mating types. To render such crosses comparable with the others, it was necessary, in the preparation of Table I, to divide by two the total number of the nectar-receiving pustules of these crosses. In the table, the crosses are, therefore, reported as if mass transfers of nectar (containing pycniospores of both (+) and (−) mating types) had been used in every case.

In the crosses, the varieties of *P. graminis* were paired in all possible combinations, that is, crosses and reciprocal crosses were made between each pair of varieties. Some of these varietal combinations were not made in sufficient number to permit a conclusive estimation of intervarietal fertility and, in general, the figures given in the last column of Table I should not be regarded as absolute values but merely as indications of fertility between the different varieties.

In connection with these crosses, two possible sources of error should be mentioned. One was the occurrence of a certain amount of fortuitous selfing, that is, the occasional, apparently spontaneous production of aecia in presumably haploid pustules that had received a transfer of nectar from another variety of the rust. Aecial pustules that apparently arose by selfing are not included in Table I. It is nevertheless possible that some instances of apparent selfing were in reality crosses in which the "female" parent race showed complete dominance. But, as progeny studies with a few cases suspected of showing complete dominance demonstrated that these were

TABLE I

FERTILITY IN CROSSES BETWEEN VARIETIES OF *Puccinia graminis*

Varieties crossed	No. pustules to which nectar applied	No. pustules that formed aecia	No. pustules of aecia that produced no infection	No. pustules of aecia regarded as hybrid	Probable percentage fertility
Tritici × *Avenae*	122	23	9	14	11–19
Avenae × *Tritici*	86	2	2	0	0– 2
Tritici × *Secalis*	17	14	0	14	82
Secalis × *Tritici*	7	4	4	0	0–57
Tritici × *Agrostidis*	34	16	1	15	44–47
Agrostidis × *Tritici*	28	0	0	0	0
Tritici × *Poae*	38	19	2	17	45–50
Poae × *Tritici*	12	1	1	0	0– 8
Avenae × *Secalis*	31	4	0	4	13
Secalis × *Avenae*	44	14	4	10	23–32
Avenae × *Agrostidis*	41	23	8	15	37–56
Agrostidis × *Avenae*	55	29	1	28	51–53
Avenae × *Poae*	47	28	10	18	39–60
Poae × *Avenae*	14	9	6	3	21–64
Secalis × *Agrostidis*	16	7	3	4	25–44
Agrostidis × *Secalis*	21	0	0	0	0
Secalis × *Poae*	27	7	3	4	15–26
Poae × *Secalis*	49	2	2	0	0– 4
Agrostidis × *Poae*	38	36	11	25	66–95
Poae × *Agrostidis*	23	20	9	11	48–87

Note: In the above crosses, nectar was transferred from many pycnial pustules of the last-mentioned variety to individual, and presumably haploid pustules of the first-mentioned. If interfertility of any two varieties were complete, all of the nectar-receiving pustules would produce aecia, and fertility (last column) would be recorded as 100%.

instances of selfing, it is unlikely that dominance was a factor of any appreciable importance in any of the crosses except possibly those between varieties *Poae* and *Agrostidis*. Another possible source of error was the presence, in certain crosses, of aecial pustules from which no uredial cultures were derived. In calculating the percentages of fertility, it was assumed that the aecia in these pustules were of hybrid origin. Where a range is given as (*Tritici* × *Avenae*, 11 to 19%) the first figure represents interfertility based on the number of aecial pustules of known hybridity, whereas the second figure is based on aecial pustules of known hybridity as well as those from which no uredial cultures were established.

The results of the crossing studies make it evident that the degree of fertility between varieties of the rust varies considerably, depending on which varieties are crossed. The crosses between varieties *Tritici* and *Secalis*, *Avenae* and

Agrostidis, *Avenae* and *Poae*, and *Agrostidis* and *Poae* were relatively high in fertility. Much lower in fertility were the crosses between varieties *Tritici* and *Avenae*, *Secalis* and *Avenae*, and *Secalis* and *Poae*.

In crosses between certain varieties, the degree of fertility varied according to the direction of the crosses. *Tritici* × *Agrostidis* crosses (*Tritici* pustules receiving *Agrostidis* nectar) indicated fertility of about 45%. The reciprocal crosses indicated complete sterility. Similarly *Tritici* × *Avenae*, *Tritici* × *Poae*, *Secalis* × *Agrostidis*, and *Secalis* × *Poae* crosses succeeded much better than the reverse crosses. The only varieties showing a high degree of interfertility, irrespective of the direction of nectar transfer, were varieties *Agrostidis* and *Poae*. A moderate interfertility seemed also to exist between these varieties and variety *Avenae* regardless of the direction of the transfer of nectar.

In all cases in which any considerable degree of sterility existed between varieties, the aecia formed were fewer in number and smaller in size than in those cases in which fertility was high. The failure to establish uredial cultures from a number of aecial pustules was largely due to the scantiness of aecial material resulting from these causes. Moreover, there appeared to be a relationship between rate of aecial development and degree of sterility between varieties—the greater the sterility the slower the development of the aecia. Where fertility was high, as in crosses between varieties *Agrostidis* and *Poae*, the rate of aecial formation was similar to that prevailing in selfing studies or in crosses involving the races of a single variety, the aecia breaking through the epidermis in six or seven days after the intermixing of nectar. In crosses between varieties *Avenae* and *Agrostidis*, in which sterility was moderate, aecia formed within a period of 7 to 13 days. In *Secalis* × *Agrostidis* crosses the period was 11 to 13 days and in *Tritici* × *Poae* crosses 9 to 16 days.

The variation that exists in the interfertility of the different varieties of *P. graminis* may possibly be interpreted as throwing some light on the relationship between varieties. Varieties *Tritici* and *Secalis* are evidently closer to each other genetically than either is to variety *Avenae*. The grass-inhabiting varieties *Agrostidis* and *Poae* seem to be closely related; and variety *Avenae* is probably more closely related to these than to the other two cereal-inhabiting rusts.

Pathogenic Characteristics of Hybrid Strains

In all crosses that resulted in the production of aecia, attempts were made to establish uredial cultures by inoculating hosts congenial to the two parent varieties used in a cross as, for instance, wheat and oats in the case of *Tritici–Avenae* crosses. If uredial cultures could be established, further infection tests were made with other cereals and, occasionally, with certain grasses. In these studies, the chief objective was to compare the host range and intensity of the pathogenicity of the hybrid rust with those of the parent rusts used in the cross. In Table II are summarized the pathogenic characteristics of a few of the hybrid rust strains as expressed in infections on wheat, oats, barley, and rye.

TABLE II

TYPES OF INFECTION PRODUCED BY INTERVARIETAL HYBRID RUSTS ON CEREAL VARIETIES AND CERTAIN GRASSES

Host variety	*Tritici* × *Avenae*		*Tritici* × *Secalis*		*Tritici* × *Agrostidis*		*Tritici* × *Poae*	*Secalis* × *Avenae*		*Avenae* × *Secalis*	
	Tr. race 36 × *Av.* race 11	*Tr.* race 39 × *Av.* race 11	*Tr.* race 1 × *Sec.*	*Tr.* race 34 × *Sec.*	*Tr.* race 17 × *Agrost.*	*Tr.* race 169 × *Agrost.*	*Tr.* race 87 × *Poae*	*Sec.* × *Av.* race 11	*Sec.* × *Av.* race 8	*Av.* race 8 × *Sec.*	*Av.* race 8 × *Sec.*
Wheat											
Little Club	0;	0;	x	x	x−	x−	x−	0;	0	0	1
Marquis	0;	0;	0;	0;	0	0;	0;	0;	0	0	0;
Kanred	0; to 2	2−	0	0	0	1−	x−	0	0	0	0;
Kota	0;	0;	0;	0;	0	0;	0	0;	0	−	0;
Arnautka	0;	0;	0;	0;	0;	0;	0	0;	−	−	0;
Mindum	0;	0;	0;	0;	1−	0;	0	0	0	0	0;
Spelmars	0;	0;	0;	0;	1	1−	0	0	−	−	−
Kubanka	0; to x	0; to 1	0;	0;	0	0;	0	0;	0	0	−
Acme	0;	0;	0	0	0	0	0	0;	0	−	0
Einkorn	x−	1	x	0;	1 to x	1−	0	1	0	0	0;
Vernal	0;	0;	0;	0;	0;	0;	0	0	0;	0	0;
Khapli	0;	0; to 1	0;	1−	0;	1=	0;	0	0	−	0;
Oats											
Victory	x	x	0	0	0	0;	0	x	x	x−	0
White Russian	2 to x	2 to x	0	−	−	0	−	x−	2±	x−	0
Richland	x	1 to x	0	−	−	0	−	x−	2 to x	x−	0
Sevnothree	2 to x	2 to x	0	−	−	0	−	x	2 to x	x−	0
Barley	2 to x	x	2 to x	2±	2 to 3	3	1 to 2	x	1 to x	x−	1+
Rye	1 to 2	1	0; to x	1−	0;	0;	1 to 2	x	x	1+	x=
Agrostis alba	−	−	−	−	0 to 2	1−	−	−	−	−	−
Poa compressa	−	−	−	−	−	0	0	−	−	−	−
Poa ampla	−	−	−	−	−	0	1 to 3	−	−	−	−

Explanation of symbols: 0 – *immunity (; following* 0 *indicates presence of small necrotic flecks);* 1 – *high resistance;* 2 – *moderate resistance;* 3 – *moderate susceptibility;* 4 – *complete susceptibility;* x – *indeterminate reaction; (+) (++) (−) (=) – indicate quantitative variations in the infection type.*

In general, it was found that hybrid cultures derived from different crosses between the same two varieties showed marked similarity in pathogenic properties. Some variation, however, did occur. For example, one of the three hybrid cultures from the crosses between *Tritici* race 36 and *Avenae* race 11 produced only necrotic flecks on Kanred wheat while the two other cultures produced a 2 type of infection. Though somewhat similar variation occurred in some other related crosses, it was rarely of such magnitude as to dispel the impression of pathogenic resemblance in cultures of similar origin.

In nearly all of the hybrid cultures there was a marked tendency for the hybrid rust to possess a wider host range than did either of the parental varieties. The extension of the host range was, however, accomplished at the expense of pathogenicity—for in no case was the pathogenicity of a hybrid rust on a given host as great as that of the parental variety that attacked that host.

Tritici–Avenae hybrid cultures combined to some degree the pathogenic powers of both parental varieties, resembling oat stem rust in moderate ability to attack oats, and wheat stem rust in slight ability to attack certain wheat varieties and barley. Despite the fact that the cultures arose from the *Tritici* side of the crosses, they resembled variety *Avenae* more than variety *Tritici*. Pathogenically, these hybrids bore a rather close resemblance to the *Tritici–Avenae* hybrid described previously by Johnson and Newton (5).

Tritici–Secalis cultures showed only a very limited pathogenicity towards wheat and rye and none at all towards oats. Barley, which is a host of both parental varieties, was attacked rather lightly. These cultures resembled rather closely certain of the *Tritici–Secalis* hybrids previously described (4).

Tritici–Agrostidis cultures showed limited ability to attack barley, wheat, and *Agrostis alba* but were not pathogenic to oats or rye. The general resemblance of these cultures to the hybrid described previously by Johnson, Newton, and Brown (4) is very close.

Most *Tritici–Poae* hybrids were virtually unable to attack wheat. The one recorded in Table II was an exception in that it produced a weak x type infection on Little Club and Kanred. Barley and rye were highly to moderately resistant. *Poa compressa*, a susceptible host of variety *Poae*, was immune but *P. ampla* varied in reaction from resistant to moderately susceptible.

Avenae–Secalis hybrids also were virtually nonpathogenic to wheat but showed some resemblance to both parental varieties by slight to moderate ability to attack oats and rye. They also possessed a limited ability to attack barley.

Hybrids between varieties *Avenae* and *Agrostidis* showed only slight ability to attack either oats or *Agrostis alba*. Pustules on oats were for the most part type 1 but a few cultures produced a weak type x. Pustules on *A. alba* never exceeded type 1.

Most *Avenae–Poae* hybrids appeared to be nearly nonpathogenic to both oats and *Poa compressa* and failed, uniformly, to attack wheat, rye, or *Agrostis alba*. One hybrid proved an exception in that it was capable of producing an x type infection on the oat varieties Victory and White Tartar. It was, however, unable to attack either *Poa compressa* or *P. ampla* and was nonpathogenic to wheat and rye.

Only scanty information is available on the pathogenicity of *Secalis–Agrostidis* hybrids. Rye and *Agrostis alba* appeared to be immune and barley highly resistant.

Varieties *Secalis* and *Poae* do not cross readily. In the four successful crosses, in which the hybrid aecia arose from *Secalis* haplonts that had received *Poae* pycniospores, rye was susceptible and *Poa compressa* moderately resistant. *P. pratensis* and *Agrostis alba* were immune as were also wheat and oats. In these crosses, the pathogenicity of the *Secalis* parent appeared to be inherited intact and to this was added a limited ability to infect *Poa compressa*.

Hybrids between varieties *Agrostidis* and *Poae* were uniformly unable to attack *Agrostis alba* but exhibited on *Poa compressa* a pathogenicity ranging from necrotic flecks and 1 type pustules to 3 type pustules—in terms of varietal reaction a range from high resistance to moderate susceptibility. *Poae* pathogenicity was therefore largely dominant to that of *Agrostidis*. Only a few of the hybrids showed any ability to attack *Poa pratensis*, and in this respect they again resembled the *Poae* parent rust, which was generally incapable of attacking that species. All of the hybrid rust cultures tested on *Poa ampla* attacked this host as would be expected since it is fully susceptible to the *Poae* variety and at least moderately so to the *Agrostidis* variety. Wheat, oats, and rye were not visibly attacked by any of the hybrids.

Inheritance of Urediospore Size and Color

The classification of *Puccinia graminis* into varieties is based on host specificity rather than on the morphology of the spores. Nevertheless, as has been clearly established by Levine (7) and Stakman and Levine (9), there are considerable differences in size and shape between the spores of certain of the varieties. The urediospores of the cereal-inhabiting varieties *Tritici* and *Avenae*, are distinctly larger than those of the grass-inhabiting varieties *Agrostidis* and *Poae* while the spores of variety *Secalis* occupy a somewhat intermediate place.

Owing to the similarity in size of the urediospores of varieties *Tritici*, *Avenae*, and *Secalis*, no attempt was made to study the inheritance of spore size in crosses between them. When, however, crosses were successfully made between any of these varieties and the smaller-spored varieties *Agrostidis* and *Poae*, comparative measurements were usually made of 10 or 20 urediospores of the hybrid and a similar number of spores of the two parent rusts.

Whenever possible the hybrid spores were compared with the actual parent strains used in the cross rather than with other accessions of the parent rust varieties.

The data thus acquired were summarized (Tables III and IV) and submitted to statistical analysis on the assumption that the combined data from crosses between any two varieties might permit generalization as to whether or not the urediospores of the hybrids were significantly different in size or shape from those of the parent rusts.

The results show that in the crosses *Tritici–Poae*, *Secalis–Poae*, *Avenae–Poae*, and *Avenae–Agrostidis* the hybrid spores are significantly different in both length and width from those of both parent varieties. Only in the *Agrostidis–Poae* crosses was there no significant size difference between hybrids and parents. Where differences existed, they were always manifested by a spore size roughly intermediate between those of the parent rusts.

In general, the hybrid urediospores did not exhibit any noticeable abnormality in form or in spore contents, but occasional hybrids showed some deviation from the normal. One cross of *Avenae* race 1 × *Poae* produced urediospores very irregular in shape and size and in organization of the spore contents. The orange pigment, which normally is present in an oval granular mass in the center of the spore, was gathered into scattered oil-like droplets or was entirely absent. In another cross of the same origin, the orange pigment was commonly gathered together into one large oil-like globule in the center of the spore.

In crosses in which the urediospore color of one parent differed from the normal, red color of stem rust, there was a strong tendency for the red color to predominate in the hybrid spores. The cross *Secalis* × *Avenae* race 4 orange produced a hybrid pathogenically much closer to the *Avenae* parent rust but with the red spore color of the *Secalis* parent. The cross *Avenae* race 6 orange × *Poae* produced urediospores that showed much of the red color of the *Poae* parent when viewed in mass. When examined with the microscope, it was seen that many spores were irregular in shape and content, some being nearly hyaline with the pigment concentrated in bright yellow droplets. Pathogenically this rust resembled the *Avenae* more than the *Poae* parent.

In one cross, *Agrostidis* × *Avenae* race 2 grayish-brown, the hybrid spores were of a color intermediate between the bright red of the *Agrostidis* rust and the dull grayish-brown of the *Avenae* parent. When viewed with the microscope, it was evident that the hybrid spores contained less orange pigment than the *Agrostidis* spores but more than those of the *Avenae* parent race.

It seems clear from the above studies that intervarietal hybrid rusts show a definite tendency to produce urediospores intermediate between those of the parent varieties in size and shape. Observable color differences between

TABLE III

FREQUENCY DISTRIBUTIONS FOR LENGTH AND WIDTH OF UREDIOSPORES OF VARIETIES OF *P. graminis* AND OF CERTAIN INTERVARIETAL HYBRIDS, WITH RELEVANT STATISTICS

Varieties	Spore classes according to: Length (μ)									Total no. spores	Statistics: Mean and S.e. of mean	Statistics: Standard deviation and S.e. of standard deviation	Statistics: Coefficient of variability and S.e. of V.
	15	18	21	24	27	30	33	36	39				
Tritici					6	3	6	4	1	20	31.65 ± 0.83	3.73 ± 0.59	11.79 ± 1.89
Avenae				11	15	21	11	2		60	28.90 ± 0.42	3.24 ± 0.30	11.21 ± 1.04
Secalis			2	12	3	2		1		20	25.35 ± 0.75	3.35 ± 0.53	13.21 ± 2.13
Agrostidis		1	16	23	8	2				50	23.64 ± 0.36	2.52 ± 0.25	10.66 ± 1.08
Poae	2	20	29	16	3					70	20.91 ± 0.32	2.68 ± 0.23	12.82 ± 1.10
Tritici × *Poae*		1	6	9	8	6				30	25.20 ± 0.61	3.34 ± 0.43	13.25 ± 1.74
Avenae × *Agrostidis*			4	23	14	7	2			50	25.80 ± 0.41	2.88 ± 0.29	11.16 ± 1.13
Avenae × *Poae*	1	7	16	19	25	16	5	1		90	25.43 ± 0.45	4.27 ± 0.32	16.79 ± 1.29
Secalis × *Poae*		4	17	11	6		2			40	23.02 ± 0.54	3.45 ± 0.39	14.99 ± 1.70
Agrostidis × *Poae*			7	9	3	1				20	23.70 ± 0.56	2.50 ± 0.40	10.55 ± 1.69
	Width (μ)												
	12	13	14	15	16	17	18	19	20	21	22				
Tritici							1	12	1	6		20	20.10 ± 0.22	0.97 ± 0.15	4.83 ± 0.76
Avenae				1	2	3	12	25	8	9		60	19.47 ± 0.17	1.30 ± 0.12	6.68 ± 0.61
Secalis			1	1	8	5	1	4				20	17.30 ± 0.30	1.36 ± 0.22	7.86 ± 1.24
Agrostidis			4	18	27			1				50	16.04 ± 0.11	0.80 ± 0.08	4.99 ± 0.50
Poae			33	26	11							70	15.19 ± 0.09	0.73 ± 0.06	4.81 ± 0.41
Tritici × *Poae*			1	8	16	2	3					30	16.43 ± 0.17	0.93 ± 0.12	5.66 ± 0.73
Avenae × *Agrostidis*			1	2	24	10	6	7				50	17.28 ± 0.17	1.20 ± 0.12	6.94 ± 0.69
Avenae × *Poae*	1	1	7	8	22	4	9	25	4	4	5	90	17.99 ± 0.24	2.29 ± 0.17	12.73 ± 0.97
Secalis × *Poae*			3	11	21	5						40	16.20 ± 0.12	0.78 ± 0.09	4.81 ± 0.54
Agrostidis × *Poae*		1	7	6	6							20	15.35 ± 0.20	0.91 ± 0.14	5.93 ± 0.94

TABLE IV

DIFFERENCES (IN MICRONS) IN MEAN SPORE LENGTH AND MEAN SPORE WIDTH OF UREDIOSPORES OF VARIETIES OF *P. graminis* AND OF CERTAIN INTERVARIETAL HYBRIDS. (THE FIGURES ABOVE THE DIAGONAL LINE REPRESENT DIFFERENCES IN MEAN SPORE LENGTH, THOSE BELOW SHOW DIFFERENCES IN MEAN SPORE WIDTH)

	Tritici	*Avenae*	*Secalis*	*Agrostidis*	*Poae*	*Tritici* × *Poae*	*Avenae* × *Agrostidis*	*Avenae* × *Poae*	*Secalis* × *Poae*	*Agrostidis* × *Poae*
	Differences in mean spore length									
Tritici		** 2.75	** 6.30	** 8.01	** 10.74	** 6.45	** 5.85	** 6.22	** 8.63	** 7.95
Avenae	* 0.63		** 3.55	** 5.26	** 7.99	** 3.70	** 3.10	** 3.47	** 5.88	** 5.20
Secalis	** 2.80	** 2.17		* 1.71	** 4.44	0.15	0.45	0.08	* 2.33	1.65
Agrostidis	** 4.06	** 3.43	** 1.26		** 2.73	* 1.56	** 2.16	** 1.79	0.62	0.06
Poae	** 4.91	** 4.28	** 2.11	** 0.85		** 4.29	** 4.89	** 4.52	** 2.11	** 2.79
Tritici × *Poae*	** 3.67	** 3.04	* 0.87	0.39	** 1.24		0.60	0.23	** 2.18	1.50
Avenae × *Agrostidis*	** 2.82	** 2.19	0.02	** 1.24	** 2.09	** 0.85		0.37	** 2.78	** 2.10
Avenae × *Poae*	** 2.11	** 1.48	0.69	** 1.95	** 2.80	** 1.56	* 0.71		** 2.41	* 1.73
Secalis × *Poae*	** 3.90	** 3.27	** 1.10	0.16	** 1.01	0.23	** 1.08	** 1.79		0.68
Agrostidis × *Poae*	** 4.75	** 4.12	** 1.95	** 0.69	0.16	** 1.08	** 1.93	** 2.64	** 0.85	
	Differences in mean spore width									

* = *Difference significant at 5% point.*
** = " " " 1% " .

varieties do not normally exist, but when they do there is a strong tendency to a complete or nearly complete dominance of normal color characteristics in the hybrid.

Progeny Studies with Intervarietal Hybrids

It has been shown (6) that, in the selfing of hybrids between two physiologic races of the variety *Tritici*, the next generation (F_2) will contain the pathogenic characters of the grandparental races in combinations that give rise to many physiologic races. Among these, both grandparental races are likely to recur.

In such crosses, the pathogenic properties appear to be transmitted as unit characters that are reshuffled to form new combinations.

If the same pattern of behavior prevailed in intervarietal crosses, it might be expected that in *Tritici–Avenae* crosses, for example, the F_2 generation would contain strains that combined in various ways the pathogenic properties of both rusts. Some strains might attack certain wheat and oat varieties heavily, whereas others might be predominantly wheat or oat strains. Possibly, even one or both of the grandparental races might occur in the F_2 generation.

Such selfing studies as have been carried out with intervarietal hybrids show a behavior differing considerably from that described above.

Two crosses of var. *Tritici* race 36 × var. *Avenae* race 11 produced practically identical results (See Table V). The F_1 hybrid rust combined to some degree the pathogenic powers of both parent rusts. It resembled oat stem

TABLE V

SUMMARY OF STUDIES ON THE SELFING OF INTERVARIETAL HYBRID RUSTS

Varieties crossed	Pathogenicity in F_1 uredial generation	Symptoms on barberry inoculated with F_1 teliospores	Pathogenicity in F_2 uredial generation
Tritici race 36 × *Avenae* race 11	Moderately pathogenic to oats and barley; slightly to wheat and rye	Small pycnia with scanty nectar. No aecia. Uredia and telia formed occasionally on old infections	Urediospores formed on barberry produced weak infection on oats; no infection on wheat
Tritici race 36 × *Avenae* race 11	As above	As above	Urediospores formed on barberry failed to cause infection
Secalis × *Avenae* race 11	Moderately pathogenic to oats, rye, and barley. Almost nonpathogenic to wheat	Small pycnia with scanty nectar. No aecia. Uredia and telia formed in one old infection	Oats highly to moderately resistant. Barley and rye highly resistant. Wheat nearly immune
Tritici race 95 × *Secalis*	F_1 identified as *Tritici* race 104, moderately pathogenic to barley and slightly so to wheat and rye	Normal pycnia and aecia	The few tests made indicated that wheat and barley were moderately resistant and rye nearly immune
Tritici race 30 × *Secalis*	F_1 identified as *Tritici* race 70, moderately pathogenic to barley and slightly so to wheat and rye	Normal pycnia and aecia	The following races of var. *Tritici* identified (number of times each identified, in brackets): 71 (4); 104 (3); 111 (1); 137 (4); 138 (7); 153 (2)
Tritici race 1 × *Secalis*	F_1 identified as *Tritici* race 103, moderately pathogenic to barley and slightly so to wheat and rye. Nonpathogenic to oats	Normal pycnia and aecia	Var. *Tritici* races 71 (1); 103 (34); 111 (10); 112 (2). All of the numbered races in the two above crosses are moderately pathogenic to barley and slightly pathogenic to wheat and rye

rust in moderate ability to attack oats, *Dactylis glomerata*, *Arrhenatherum elatius*, and *Lolium perenne*. It resembled wheat stem rust in slight ability to attack certain wheat varieties and barley. Despite the fact that the rust originated from the *Tritici* side of the cross, it resembled oat stem rust in pathogenicity more than wheat stem rust.

Selfing studies with the two hybrid rusts produced identical results. On barberry, both produced small pustules of pycnia with only a trace of nectar in which, however, a considerable number of pycniospores were present. No aecia were formed in consequence of intermixing this nectar, but urediospores and teliospores occurred in a few of these pustules between two and three months after infection.

In several attempts to produce infection on cereals with the urediospores just mentioned, only one positive result was obtained—a weak infection of Victory oats. The urediospores differed in several respects from those of normal oat stem rust: they were less regular in shape and size, with many shrunken, colorless spores as well as a high proportion of spores containing one or more yellow, oil-like droplets in the center of the spore instead of the granular yellow mass characteristic of normal rust spores.

It seems probable that the pycniospores produced by the *Tritici–Avenae* hybrid are functionless as the application of the scanty pycnial nectar to haploid pustules of var. *Avenae* race 11 failed to produce aecia in these pustules. The failure of the hybrid race itself to produce aecia in repeated selfings may not be solely due to lack of function on the part of the pycniospores as liberal applications of nectar of var. *Avenae* races 1 and 12 and of var. *Tritici* race 15 to the pycnia of the hybrid likewise failed to produce aecia.

Very similar results were secured in the selfing of the F_1 hybrid of the cross var. *Secalis* × var. *Avenae* race 11. The hybrid rust, which arose from the *Secalis* side of the cross, had some of the pathogenic powers of both parent rusts but resembled oat stem rust more than rye stem rust. Numerous infections were obtained on barberry with teliospores formed on oats and barley. The pycnia produced scarcely any nectar and no aecia resulted from the intermixing of such nectar as was present. Uredia, and subsequently telia, were formed in one infection about two months after inoculation. The urediospores infected Victory oats lightly (x type infection) and other oat varieties still more lightly. Barley was moderately to highly resistant; rye highly resistant; and wheat virtually immune.

The selfing of F_1 hybrids of crosses between varieties *Tritici* and *Secalis* has produced notably different results. The hybrids that have been studied have produced normal pycnia and aecia on barberry and no indications of sterility have been noted.

On cereals, the pathogenicity of the progeny of the three *Tritici* × *Secalis* crosses studied has been rather strikingly similar. In each case, the F_1 uredial generation was moderately pathogenic to barley and, in general, only slightly pathogenic to wheat and rye.

It might have been expected that in the F_2 uredial generation (resulting from the selfing of the F_1 hybrid) some rather typical wheat stem rust and rye stem rust would have appeared as well as strains similar to those that comprised the F_1 generation. Actually, the pathogenic variation in F_2 was rather narrowly limited to strains of rust that bore a close resemblance to the F_1 generation, and no pathogenically vigorous strains of either wheat stem rust or rye stem rust could be discovered.

The results in the F_2 generation of the cross *Tritici* race 1 × *Secalis* are typical of the crosses that have been studied in the F_2 generation (Table VI).

TABLE VI

PATHOGENICITY ON WHEAT, BARLEY, AND RYE OF RACES OCCURRING IN F_1 AND F_2 GENERATIONS OF THE CROSS VAR. *Tritici* RACE 1 × VAR. *Secalis*

Variety tested	Parent races		F_1	Physiologic races in F_2			
	Tritici race 1	*Secalis*	Race 103	71	103	111	112
Little Club	4	0;	x+	3+	x+	3±	4
Marquis	4−	0	0;	x−	0	1−	x
Kanred	0	0	0	0	0;	0	0
Kota	3+	0	0;	0;	0	0;	0;
Arnautka	1=	0	0;	0;	0	0;	0;
Mindum	1	0	0;	1=	0;	0;	0;
Spelmars	1=	0	0;	0;	0;	0;	0;
Kubanka	3+	0	0;	0;	0;	0;	x−
Acme	3+	0	0	0;	0;	0;	0;
Einkorn	3	0	2−	1	2−	1−	3
Vernal	0;	0	0;	0;	1=	0;	0;
Khapli	1−	0	0;	0;	0;	1−	1−
Barley vars.	3−	3−	2−3	1−3	2−3	2−3	3
Rye vars.	0;	4	1−3	0;	1−3	1−3	1

Explanation of symbols:

0 – *immunity (; following* 0 *indicates presence of small necrotic flecks)*
1 – *high resistance*
2 – *moderate resistance*
3 – *moderate susceptibility*
4 – *susceptibility*
x – *indeterminate reaction*
(+) (++) (−) (=) – *indicate quantitative variations in the infection type*

All cultures derived from the aeciospores were very similar irrespective of whether they were established on wheat or rye. Though identifiable as different physiologic races, they were either pathogenically identical with the F_1 hybrid, which had been classified as race 103 of var. *Tritici*, or they differed only slightly from it. The cultures established on Little Club wheat were identified as race 103 (15 cultures); race 111 (6 cultures); and race 112 (1 culture). Those established on Rosen rye were identified as race 71 (1 culture); race 103 (9 cultures); race 111 (4 cultures); and race 112 (1 culture).

These races, which occur rarely if at all in field collections, had all been found previously in the F_1 generation of *Tritici–Secalis* crosses and may therefore be regarded as characteristic progeny of such crosses.

Although the races shown in Tables VI and VII are all assigned to var. *Tritici*, the propriety of doing so is rather doubtful as, with the exception of

TABLE VII

PATHOGENICITY ON WHEAT, BARLEY, AND RYE OF RACES OCCURRING IN F_1 AND F_2 GENERATIONS OF THE CROSS VAR. *Tritici* RACE 30 × VAR. *Secalis*

Variety tested	Parent races		F_1	Physiologic races in F_2					
	Tritici race 30	*Secalis*	Race 70	71	104	111	137	138	153
Little Club	4	0;	3+	3	4	3±	4	2	3±
Marquis	4	0	x+	x−	x=	1−	4−	1−	1+
Kanred	0	0	0	0	0	0	4−	0	3−
Kota	3+	0	0;	0;	0;	0;	0;	0;	0;
Arnautka	x	0	0;	0;	0;	0;	1−	0;	0;
Mindum	x	0	0;	1=	0;	0;	1−	0;	0
Spelmars	x	0	0;	0;	0;	0;	1−	0;	0
Kubanka	x	0	0;	0;	0;	0;	3	0;	0;
Acme	x	0	0;	0;	0;	0;	1−	0;	0
Einkorn	3+	0	1=	1	3+	1−	3−	1+	0;
Vernal	4=	0	x	0;	x+	0;	x−	x−	x−
Khapli	1	0	1=	0;	0;	1−	1	0;	0;
Barley vars.	3	3−	3	1−3	1−3	2−3	2−3	2−3	−
Rye vars.	0;	4	1	0;	1−3	1−3	1−3	1−3	−

race 137, their pathogenicity to wheat varieties is extremely limited. None of the 12 differential hosts is fully susceptible to race 103 or race 111; only one variety (Little Club) is fully susceptible to race 71; and only two (Little Club and Einkorn) are susceptible to race 112. Many other wheats susceptible to most physiologic races of var. *Tritici* were tested and found to be moderately or highly resistant to these races. The pathogenicity of these races towards rye is even more limited as the variety most severely attacked (Rosen) cannot be regarded as fully susceptible. Barley, which is a common host of both the parental rusts used in the cross (var. *Tritici* and var. *Secalis*), is probably a more congenial host to the races occurring in the F_1 and F_2 generations of this cross than either wheat or rye. The ability of these races to attack barley and their limited pathogenicity towards wheat and rye may probably be interpreted as proof that the hybrid progeny is pathogenically intermediate between wheat stem rust and rye stem rust.

The absence, in the F_2 generation of *Tritici-Secalis* crosses, of the grandparental wheat stem rust races or of typical rye stem rust does not necessarily prove that they never occur as segregants from similar crosses. That rather typical *Tritici* races may occur in F_2 is suggested by the presence in the F_2 generation of the cross *Tritici* race 30 × *Secalis* of race 137, which has a

sufficiently wide pathogenic range on wheat varieties to be accepted as a fairly typical wheat stem rust race (Table VII). The rarity of typical *Tritici* strains and the absence of *Secalis* strains in the F_2 generations of the crosses studied does, however, suggest the presence of some condition that prevents their appearance. Certain characteristics of the *Tritici* races used in the crosses failed completely to appear in the F_2 generation. For example, all three of the *Tritici* parent races (races 1, 30, and 95) were highly pathogenic to the wheat variety Acme. None of the F_2 progeny showed any appreciable degree of pathogenicity on this variety. The moderate ability of race 30 to attack the durum wheats Arnautka, Mindum, Spelmars, and Kubanka did not appear at all in the F_2 generation of the cross *Tritici* race 30 × *Secalis*. On the other hand, there occurred in the F_2 generation of this cross a wheat stem rust characteristic not shown by the parent *Tritici* race. Race 30, the *Tritici* parent, does not attack the wheat variety Kanred. The F_2 generation, however, contained race 137, which attacks Kanred vigorously, and race 153, which attacks it moderately. Presumably, the occurrence in F_2 of ability to attack Kanred is due to heterozygosity of the race 30 parent for this character, which is known to behave as a recessive.

The reason for the nonappearance in F_2 of some of the chief pathogenic characteristics of the parental rusts is not at all clear. It is not surprising that strains identical to the wheat stem rust and rye stem rust entering into the crosses should not appear in the relatively small F_2 populations studied. As the genotype of each of the parents is made up of many factors, it is not to be expected that those identical factorial combinations would recur frequently in F_2. The great majority of the F_2 combinations would be heterozygous for several *Tritici* and *Secalis* factors. Those that approach homozygosity for, say, *Tritici* factors might contain *Secalis* genes or chromosome segments in a homozygous condition that would exclude from the genotype the corresponding *Tritici* genes or segments. A genotype unbalanced or deficient with respect to the *Tritici* complement might result in nonviability, the effect of which would be the infrequent appearance of true *Tritici* types. It is true that such genotypic unbalance might also occur in the more heterozygous combinations but, as these make up the bulk of F_2 combinations, the effect on their frequency of appearance would be less evident.

Another possible explanation involves the relationship of nuclei and cytoplasm. As nuclei and cytoplasm both affect pathogenicity, possibly the reason is to be found in some interaction between the two. It may be that, in hybrids arising from *Tritici* haplonts, the cytoplasm in which the nuclei operate is chiefly of *Tritici* origin. If so, there might be a tendency in such crosses towards favoring the expression of *Tritici* characteristics in both F_1 and F_2 and a corresponding tendency towards partial inhibition of the expression of *Secalis* characteristics. In hybrids arising from *Secalis* haplonts, this tendency would presumably be reversed. Such an assumption would account for the failure of typical rye stem rust to appear in the F_1 and F_2

generations of the crosses in which the hybrids originated from the mycelia of *Tritici* haplonts, but it would not account for the absence of the *Tritici* characteristics mentioned above. Only a comparative study of reciprocal (*Secalis* × *Tritici*) crosses could test this idea, and, so far, no such study has been made.

Discussion

The word "specialization", as applied to a living organism, connotes modification, physiological or morphological or both, in the organism. Regarded from the standpoint of evolution, it implies a divergent development in which different lines of the same organism acquire somewhat different ways or habits of life that eventually become fixed modifications. In *Puccinia graminis*, the adaptation of different lines or clones of the organism to different grass hosts is probably of long standing and has led to the division of the organism into rather narrowly specialized groups. These groups, *formae speciales* or varieties, still maintain contact with each other through their common ability to parasitize the barberry.

If it is granted that the different varieties of *P. graminis* had a common origin, it follows that the species was originally readily capable of forming new binuclear relationships on the barberry. As shown in the present paper, this does not hold true for the species as we know it to-day. Some of the varieties here discussed do not readily combine to form dikaryons. If the establishment of such dikaryons can be regarded as hybridization, then any obstacle to their establishment is to be looked upon as intervarietal sterility, and as interfertility between organisms is a sign of closeness in relationship so is intersterility a sign of divergence. Viewed thus, it is possible to group the varieties on the basis of relationship. *Agrostidis* and *Poae* form one pair of closely related varieties and *Tritici* and *Secalis* form another such pair. In both these pairs, there is confirmatory evidence to be found in their pathogenic behavior. Varieties *Agrostidis* and *Poae* have a common host in *Poa ampla*, and varieties *Tritici* and *Secalis* in barley and a number of grasses. Variety *Avenae* appears to lack close relationship with any of the other four varieties despite the fact that it can form dikaryons with all of them.

Further evidence of the lack of relationship between var. *Avenae* and vars. *Tritici* and *Secalis* is to be found in the behavior of the hybrids on the alternate host, barberry. The hybrid teliospores of *Tritici* × *Avenae* and *Secalis* × *Avenae* are capable of germination and their sporidia infect barberry readily. That the haploid mycelia grow quite readily is shown by the large size of many of the infections. Sterility, however, results from the fewness of the pycnia formed and their evident lack of ability to produce an abundance of pycnial nectar and possibly also from the failure of the pycniospores to function normally.

The pathogenicity of the F_1 and F_2 hybrids is of interest alike from the practical and the theoretical viewpoint. In any intervarietal hybrid, the F_1 aeciospore and urediospore contains two nuclei, one derived from each parental

variety. Hence it is not unexpected that the hybrid rust should show some of the characteristics of each of the parents. Complete dominance of the pathogenicity of one or the other parent is probably rare but it is not uncommon, as in *Tritici* × *Avenae* hybrids, for the hybrid to resemble one rust much more than the other. The factors governing pathogenicity in F_1 are probably mostly nuclear as in *Tritici* × *Avenae* crosses the *Avenae* characters predominate despite the fact that the hybrids arose from fertilized *Tritici* haplonts. If the cytoplasm were important in this respect, it would be expected that *Tritici* characters would predominate as the hybrids presumably contain mostly *Tritici* cytoplasm.

In connection with the F_2 generation there is some evidence, derived from *Tritici* × *Secalis* crosses, that variation is limited and that the F_2 variants bear a rather close pathogenic resemblance to the F_1. It may not be safe to generalize from the rather limited studies carried out thus far; but, if this type of variation should be common in intervarietal hybridization, it is clear that it would constitute a factor favoring the creation of new pathogenic types of rust and would be a stabilizing influence tending to perpetuate the type created by the cross.

The results of surveys carried out over many years on the specialization of wheat stem rust, oat stem rust, and, to a certain extent, rye stem rust do not indicate that the creation of new strains of rust through intervarietal hybridization is of frequent occurrence or of much practical importance. These rusts are rather well defined pathogenically and there is rarely any difficulty in assigning any given collection of a rust to one or the other. The work here described indicates that there are two factors that render natural hybridization between the varieties of *P. graminis* of little practical importance. One of these is intervarietal sterility, which militates against the creation of hybrids. The other is the fact that the broadening of the range of pathogenicity, which is so characteristic of intervarietal hybrids, is commonly accompanied by a reduction in pathogenic intensity on any given host.

Acknowledgments

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References

1. Craigie, J. H. Experiments on sex in rust fungi. Nature, 120 : 116-117. 1927.
2. Craigie, J. H. Discovery of the function of the pycnia of the rust fungi. Nature, 120 : 765-767. 1927.
3. Craigie, J. H. An experimental investigation of sex in the rust fungi. Phytopathology, 21 : 1001-1040. 1931.
4. Johnson, T., Newton, Margaret, and Brown, A. M. Hybridization of *Puccinia graminis Tritici* with *Puccinia graminis Secalis* and *Puccinia graminis Agrostidis*. Sci. Agr. 13 : 141-153. 1932.

5. JOHNSON, T. and NEWTON, MARGARET. Hybridization between *Puccinia graminis Tritici* and *Puccinia graminis Avenae*. World's Grain Exhibition. Conf., Proc. II : 219-223. 1933.

6. JOHNSON, T. and NEWTON, MARGARET. Mendelian inheritance of certain pathogenic characters of *Puccinia graminis Tritici*. Can. J. Research, C, 18 : 599-611. 1940.

7. LEVINE, M. N. A statistical study of the comparative morphology of biologic forms of *Puccinia graminis*. J. Agr. Research, 24 : 539-567. 1923.

8. LEVINE, M. N. and COTTER, R. U. A synthetic production of *Puccinia graminis Hordei* F. and J. Phytopathology, 21 : 107. 1931.

9. STAKMAN, E. C. and LEVINE, M. N. *Puccinia graminis Poae* Erikss. and Henn. in the United States. J. Agr. Research, 28 : 541-548. 1924.

10. STAKMAN, E. C., LEVINE, M. N., and COTTER, R. U. Origin of physiologic forms of *Puccinia graminis Tritici* through hybridization and mutation. Sci. Agr. 10 : 707-720. 1930.

11. TETEREVNIKOVA-BABAYAN, D. N. Observations on biological races of *Puccinia graminis* Pers. at Detskoye Selo in 1926 and 1927. Morbi Plant. 17 : 35-50. 1928.

TEMPERATURE AND CULTURAL STUDIES ON *UROCYSTIS TRITICI* KOERN.[1]

By Yu-san Wu[2]

Abstract

A study of the effect of temperature on the infection of wheat by *Urocystis tritici* was made using Petri dishes in incubators held at various temperatures from 9° to 26° C. The seedlings were transferred to pots as soon as the plumules broke through the coleoptiles. The percentages of infection obtained at various temperatures were as follows:—10.8% at 9° to 14° C., 13.8 at 16°, 21.1 at 18°, 26.1 at 20°, 15.7 at 22°, 6.9 at 24°, and 3.0% at 26° C. Growth studies of three geographical isolates of *U. tritici* were made on 12 different artificial media. Cultures were obtained by plating bits of disinfected unbroken, fresh, smutted plant tissue. The smut organism was found to be culturable on various media, though its growth rate was rather slow. The three isolates grew well on potato dextrose agar, Thaxter's hard agar, 4% sucrose potato agar, wheat seedling agar, and Czapek's synthetic medium, and were found to differ characteristically on five different media.

Introduction

Flag smut of wheat is a very important disease in China, especially in the northern wheat growing regions. In general, it causes severe damage to winter wheat, and as a rule is less severe on spring sown wheat. The losses caused by the smut of wheat in 1936 and 1937 were estimated to be 5.0% and 4.0% to 5.0%, respectively.

Part I. Temperature Studies

Previous Work

Griffiths (6) in 1924 reported that infection occurred at 6° to 12° C., 17° to 19° C., and 21.5° to 23.5° C., but did not occur at 25° to 26.6° C. The highest percentage of infection that she obtained was at 21.5° to 23.5° C. Noble (7) stated in 1924 that the optimum soil temperature for infection of *Urocystis tritici* Koern. ranged from 14° to 21° C. The most severe and, at the same time, the earliest recorded infection occurred on plants kept at 19° to 21° C. Infection also took place at 23° to 25° C., but no infection occurred at 29° to 31° C. In an experiment made by Verwoerd (10) in 1929 infection was secured at 22° C., but no infection occurred at a temperature of 31° C. In 1933, Faris (4) similarly reported that high infection occurred at 10° to 20° C., and also that the optimum temperature could be shifted by the soil moisture content.

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Material and Methods

Thoroughly mixed sandy loam, obtained from the field, was run through a sieve, and then sterilized by hot air for an hour at a temperature of 160° C. Fifty grams of this dry soil was placed in sterilized Petri dishes. Ten cc. of sterile tap water was added to each dish except in Experiment I, to which 15 cc. of water was added. The seeds were disinfected by soaking in aqueous bleaching powder solution (50% by weight of commercial powder) for 20 min. and washing repeatedly with sterilized water. They were then moistened with a wet cloth so that a large quantity of smut spores would adhere to them. Each Petri dish contained 60 seeds in Experiment I, and 50 in Experiments II, III, and IV. The dishes were then placed at various temperatures in incubators. As soon as the plumules broke through the coleoptiles, the germinating seeds were planted in 8-in. pots, 40 to 50 per pot, at a depth of half an inch below the soil surface as suggested by Angell (1, 2), in order to prevent tillering and to produce symptoms of the disease as early as possible. Temperature fluctuation in each incubator was kept within a range of $\pm$ 1° C.

The smut was collected from the experiment field in Nanking. A very susceptible variety of wheat, H1102, was used in all experiments. Plants were grown in the greenhouse. The experiment was made in the winter of 1935 and repeated in the winter of 1936. Records were taken when the plants were blossoming.

Results and Conclusions

The results of the experiment are shown in Table I. The temperature range of infection was from 9° to 26° C., with the optimum at 20° C. The rate of infection was, however, greatly decreased near the two extremes.

TABLE I

THE PERCENTAGE OF INFECTION OF *Urocystis tritici* ON WHEAT UNDER VARIOUS SOIL TEMPERATURES IN 1935 AND 1936

Temperature in degrees centigrade	Experiments in 1935						Experiments in 1936						Average infection in %
	I			II			III			IV			
	Total plants	Smutted plants	Infection in %	Total plants	Smutted plants	Infection in %	Total plants	Smutted plants	Infection in %	Total plants	Smutted plants	Infection in %	
9-14	116	12	10.4	—	—	—	83	11	13.2	93	8	8.6	10.8*
16	—	—	—	—	—	—	84	13	15.5	75	9	12.0	13.8**
18	116	20	17.2	94	3	3.2	89	30	33.5	76	23	30.3	21.1
20	119	31	26.1	97	4	4.1	95	40	42.1	72	23	31.9	26.1
22	102	12	11.8	96	2	2.1	87	21	24.1	65	16	24.6	15.7
24	107	5	4.7	96	3	3.1	96	13	13.5	65	4	6.1	6.9
26	103	3	2.9	96	1	1.1	78	5	6.4	65	1	1.5	3.0

* *The average of three tests.*

** *The average of two tests.*

The results thus obtained are quite similar to those reported by previous workers. It appears that the optimum soil temperature for the infection of flag smut of wheat is approximately 20° C., and probably this is the explanation for winter wheat being more highly infected than spring sown wheat, because, ordinarily, the soil is warmer when the winter wheat is being sown.

Part II. Cultural Studies

Although many smut fungi have been induced to grow in pure culture, attempts to grow the organism causing flag smut of wheat in pure culture have not hitherto met with success. The first reported attempt to culture *U. tritici* on artificial media was made by Verwoerd (10) in 1929, but was unsuccessful. In 1937 and 1938, the writer tried to culture this fungus, with the results herein reported.

Materials and Methods

Flag smut isolates were made in 1937 and 1938. The causal organism was isolated in pure culture in 1937 at Nanking from Kaifeng and from Hsuchow collections and again in 1938 at Chengtu, Szechuan, from Tsetung collections. The isolation procedure consisted of cutting half centimeter portions of unbroken fresh smutted tissue from smutted plants, soaking these bits of tissue in a 20% solution of commercial bleaching powder or in mercuric chloride solution (1-1000) for 10 min., rinsing in sterile water, and then transferring to culture tubes. Eleven kinds of organic media and one synthetic medium were used. Fifty cc. Erlenmeyer flasks with 25 cc. nutrient media added were used for cultural purposes. These flasks were autoclaved under a pressure of 15 lb. for half an hour. Transfers were made with a loop needle using 37-day-old cultures as sources of inoculum. Each kind of medium was triplicated. After 60 days, the results were recorded.

The description of growth characteristics includes diameter of colony, coloration, topography, consistency, surface, zonation, and margin, following the method used by Christensen and Stakman (3), Rodenhiser (9), and Ficke and Johnston (5) to describe smut growth on media. The nomenclature of coloration was according to Ridgway's *Color standards and color nomenclature* (8).

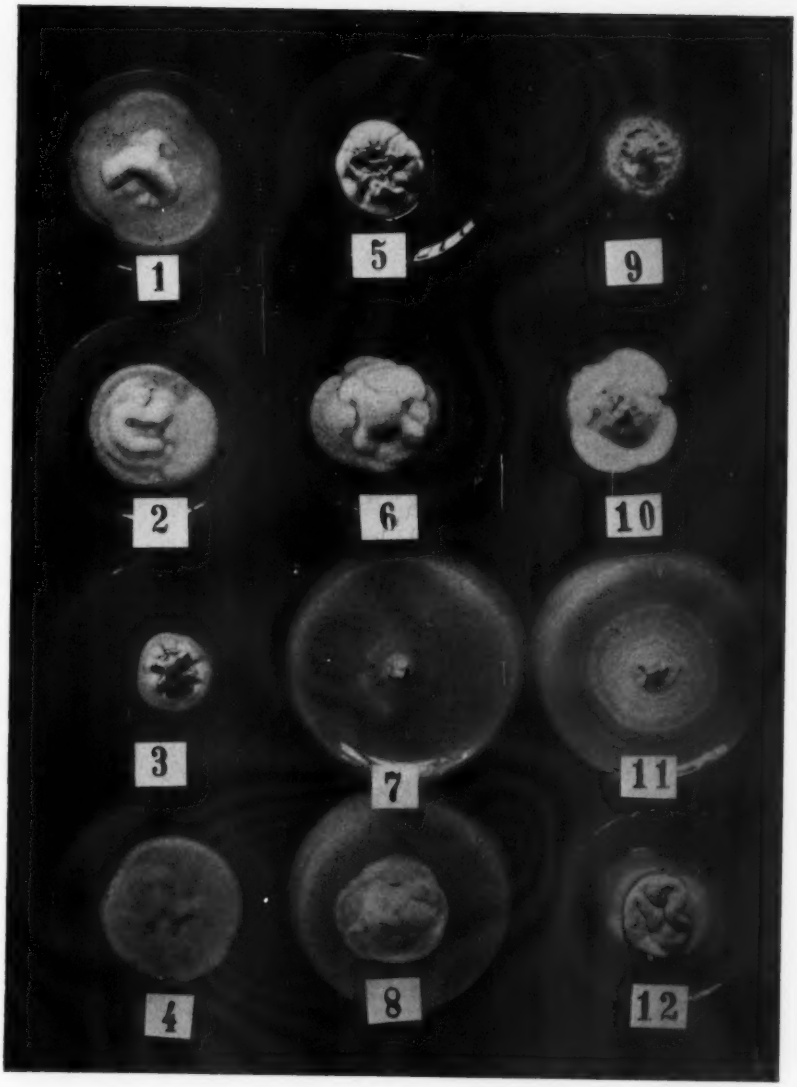
Results

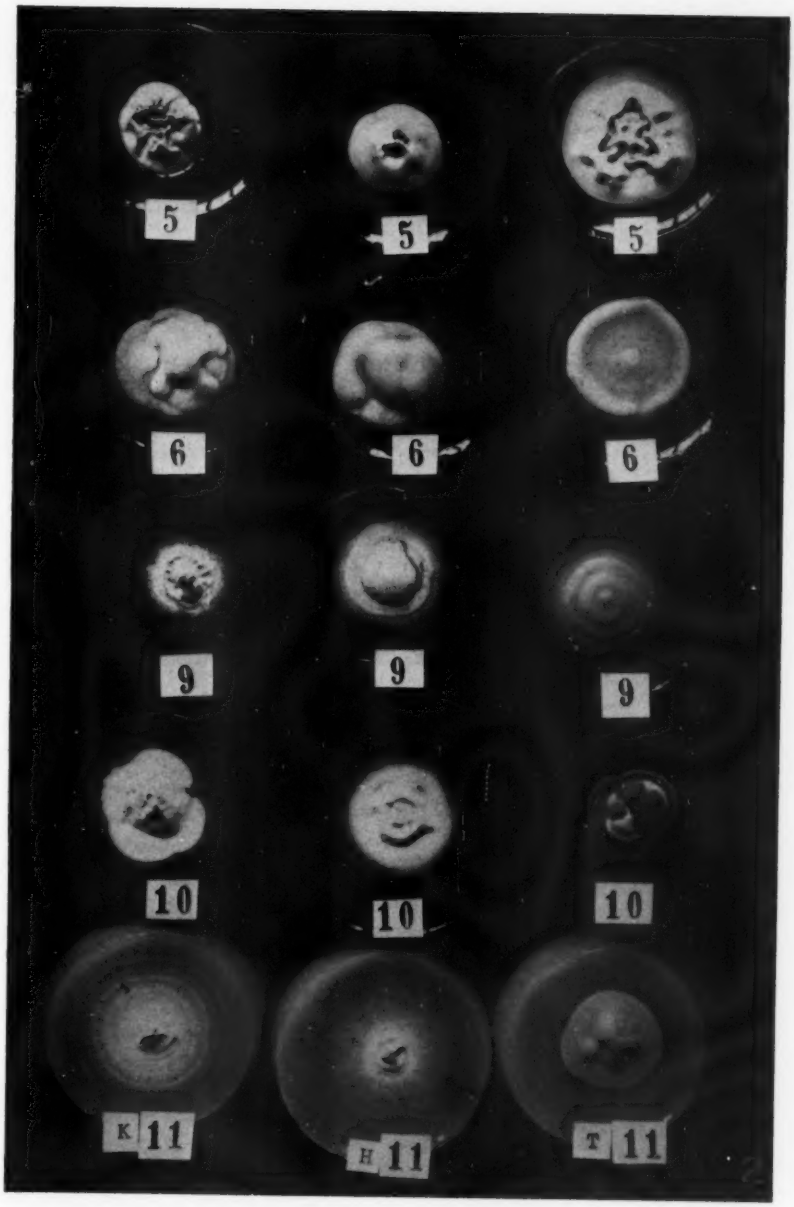
Cultures from Kaifeng, Hsuchow, and Tsetung grew well on the 12 media, especially on potato dextrose agar, Thaxter's hard agar, 4% sucrose potato agar, wheat seedling decoction agar, and Czapek's synthetic medium.

The characteristics of the Kaifeng cultures on 60-day-old potato dextrose agar medium were as follows:—26.1 mm. in average diameter; white to dark olive, or buff in coloration; raised umbonate in topography; coriaceous and

FIG. 1. *The cultural characteristics of 60-day-old colonies of the Kaifeng isolate on various agar media: (1) potato dextrose, (2) Thaxter's hard, (3) 2% maltose potato, (4) 4% sucrose potato, (5) 2% malt extract, (6) 2% malt extract plus 1% dextrose, (7) corn meal dextrose, (8) oatmeal dextrose, (9) carrot decoction, (10) wheat seedling decoction, (11) wheat meal dextrose, and (12) Czapek's synthetic.*

PLATE I





See p. 69 for caption.

mycelioid in consistency; velvety surface; without zonation; lacerate, lobate, or entire and mycelioid, appressed on margin. The important cultural characteristics of Kaifeng cultures on various media are summarized in Table II and shown in Fig. 1.

A comparative study of cultural characteristics of these cultures (Kaifeng, Hsuchow, and Tsetung) on five kinds of artificial media was also made. The results are summarized in Table III. Photographs of 60-day-old colonies are shown in Fig. 2.

The outstanding differences on various media are as follows:—

1. On 2% malt extract agar.

The Kaifeng isolate was usually tilleul buff, vinaceous buff to white colored in center, verrucose with furrows leading to the edge in topography, and lobate on the margin. The Hsuchow isolate was white and verrucose at the center; other parts of the colony were deep olive buff and smooth. The Tsetung isolate was generally white with tilleul buff ridges and a pale olive buff, entire margin.

2. On 2% malt extract plus 1% dextrose agar.

In regard to color, the Kaifeng isolate was pale smoke gray to smoke gray in some portions, the Hsuchow isolate was olive buff, and the Tsetung isolate deep olive buff. As to topography, the Kaifeng isolate was umbonate and warty; the Hsuchow isolate was pulvinate; and the Tsetung isolate was smooth with convex center. The margin was usually lobate in the Kaifeng isolate, and entire in the others.

3. On carrot decoction agar.

The Kaifeng isolate was pale brownish drab in the central portion and white in other parts, verrucose in topography, and erose on the margin. The Hsuchow isolate was white to pale olive buff in color with entire margin, while the Tsetung isolate was characterized by a light buff color, the presence of zones, and the lacerate, raised margin.

4. On wheat seedling decoction agar.

The chief variation of these three isolates on this medium lay in the coloration, which was pale smoke gray in the Kaifeng isolate, white in the Hsuchow, and tricolor in the Tsetung.

5. On wheat meal dextrose agar.

The Kaifeng isolate was pallid brownish drab in the central portion, the Hsuchow isolate was white in color, and the Tsetung was pale olive buff. As to topography, it was rugose in the Kaifeng isolate, convex in

FIG. 2. *The comparative cultural characteristics of 60-day-old colonies of the Kaifeng (K), Hsuchow (H), and Tsetung (T) isolates on various agar media. (5) 2% malt extract, (6) 2% malt extract plus 1% dextrose, (9) carrot decoction, (10) wheat seedling decoction, and (11) wheat meal dextrose.*

TABLE II

CULTURAL CHARACTERISTICS OF 60-DAY-OLD COLONIES OF THE KAIFENG ISOLATE ON 12 MEDIA

Media	Characteristics			
	Av. diameter* colony in mm.	Coloration	Topography	Margin
Potato dextrose agar	26.1	Umbonate white; other part dark olive to olive buff	Raised; umbonate	Lobate; lacerate, or entire mycelioid; appressed
Thaxter's agar	21.2	Dark olive buff, or citrine drab; white in verrucose part	Raised; verrucose	Slight lacerate; mycelioid; depressed
2% Maltose potato agar	14.1	Brownish drab in central portion, white in the other part	Raised; umbonate; rugose with furrows	Lacerate; mycelioid; appressed
4% Sucrose potato agar	26.4	White to olive buff; verrucose part white	Raised; verrucose	Slight lacerate to lacerate; appressed
2% Malt extract agar	15.6	Tilleul buff; vinaceous buff to white; verrucose part white to pale olive buff	Raised; verrucose to rugose	Lobate; mycelioid; appressed
2% Malt extract plus 1% dextrose agar	23.2	Some part pale smoke gray to smoke gray; other part pale olive buff	Raised; umbonate; warty	Lobate; mycelioid; appressed
Corn meal dextrose agar	17.2	Pale smoke gray to smoke gray	Raised; slight umbonate	Lacerate; mycelioid; papery appressed
Oatmeal dextrose agar	17.5	Pale olive buff; some portions pale smoke gray	Raised; verrucose	Lacerate; mycelioid; appressed
Carrot decoction agar	18.0	Central part pale brownish drab; other part white	Raised; umbonate; verrucose	Erose; mycelioid; appressed
Wheat seedling decoction	19.8	Pale smoke gray	Raised; verrucose	Lobate; mycelioid; appressed
Wheat meal dextrose agar	20.1	Central part pallid brownish drab; other part white	Raised; rugose in center	Entire; mycelioid; appressed
Czapek's synthetic agar medium	20.0	White to pale olive buff	Raised; verrucose to pulvinate at center	Lacerate; mycelioid to bacterioid; appressed

* *Each figure is the average diameter of three separate colonies.*

TABLE III

COMPARATIVE CULTURAL CHARACTERISTICS OF 60-DAY-OLD COLONIES OF THE KAIFENG (K), HSUCHOW (H), AND TSETUNG (T) ISOLATES ON FIVE AGAR MEDIA

Media	Characteristics											
	Average diameter* of colony in mm.			Coloration			Topography			Margin		
	Collections											
	K.	H.	T.	K.	H.	T.	K.	H.	T.	K.	H.	T.
2% Malt extract agar (5)	15.6	17.2	25.8	Tilleul buff, vinaceous buff to whitish; verrucose part white to pale olive buff	Center white; other part deep olive buff	White; ridges tilleul buff; margin pale olive buff	Raised; verrucose to rugose	Raised, smooth; verrucose in center	Raised; verrucose with depressed pits at center	Lobate; mycelioid; appressed	Slight lacerate; mycelioid; appressed	Entire; mycelioid; appressed
2% Malt extract plus 1% dextrose agar (6)	23.2	21.3	22.5	Some portions pale smoke gray to smoke gray; other part white to pale olive buff	Olive buff to deep olive buff	Olive buff	Raised; umbonate; warty	Raised; pulvinate	Raised; smooth; center convex	Lobate; mycelioid; appressed	Entire or lacerate; mycelioid; raised	Entire mycelioid; raised
Carrot decoction agar (9)	18.0	23.7	24.0	Central part pale brownish drab; other part white	White to pale olive buff	Light buff	Raised; umbonate, verrucose	Raised; umbonate	Raised; umbonate	Erose; mycelioid; appressed	Entire; mycelioid; appressed	Lacerate; mycelioid; raised
Wheat seedling decoction agar (10)	19.8	23.6	18.2	Pale smoke gray	White	Verrucose wood brown; some portions white; deep olive buff periphery	Raised; verrucose	Center raised; slight verrucose or rugose	Raised; verrucose	Lobate; mycelioid; appressed	Lacerate; mycelioid; appressed	Lacerate; mycelioid; raised
Wheat meal dextrose agar (11)	20.1	26.5	16.0	Central part pallid brownish drab; other part white	White	Pale olive buff	Raised; rugose in the central part	Raised; center convex	Raised; center umbonate; warty	Entire; mycelioid; appressed	Erose; mycelioid; appressed	Entire; mycelioid; raised

* *Each figure is the average diameter of three separate colonies.*

the Hsuchow, and umbonate and warty in the Tsetung. The margin was usually entire in the Kaifeng isolate, erose in the Hsuchow, and entire and raised in the Tsetung. In addition, the Kaifeng isolate had concentric zonation.

Conclusions

The foregoing results showed that *U. tritici*, like other smut fungi, could be cultured on various media, although its growth rate was rather slow. The three geographical isolates differed characteristically on five different media.

Acknowledgments

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References

1. ANGELL, H. R. A preliminary note on the recognition of flag smut or bunt infection based on the deformation of seedlings. J. Australian Council Sci. and Ind. Research, 7 : 110-112. 1934.
2. ANGELL, H. R. Flag smut of wheat—early symptoms. J. Australian Council Sci. and Ind. Research, 7 : 153-156. 1934.
3. CHRISTENSEN, J. J. and STAKMAN, E. C. Physiologic specialization and mutation in *Ustilago zeae*. Phytopathology, 16 : 979-999. 1926.
4. FARIS, J. A. Influence of soil moisture and soil temperature on infection of wheat by *Urocystis tritici*. Phytopathology, 23 : 10-11. 1933 (Abstract).
5. FICKE, C. H. and JOHNSTON, C. O. Cultural characteristics of physiologic forms of *Sphacelotheca sorghi*. Phytopathology, 20 : 241-249. 1930.
6. GRIFFITHS, MARION A. Experiments with flag smut of wheat and the causal fungus, *Urocystis tritici* Kcke. J. Agr. Research, 27 : 425-449. 1924.
7. NOBLE, R. J. Studies on the parasitism of *Urocystis tritici* Koern., the organism causing flag smut of wheat. J. Agr. Research, 27 : 451-489. 1924.
8. RIDGWAY, R. Color standards and color nomenclature. A. Hoen & Company, Baltimore. 1912.
9. RODENHISER, H. A. Physiologic specialization in some cereal smuts. Phytopathology, 18 : 955-1003. 1928.
10. VERWOERD, L. The biology, parasitism and control of *Urocystis tritici* Koern., the causal organism of flag smut in wheat (*Triticum* spp.) and recording the occurrence of *Urocystis occulta* (Wallr.) Rab. in South Africa as the cause of "Stem smut" in rye. South Africa Dept. Agr. Sci. Bull. 76. 1929. (Dutch with English translation.)

THE CHEMICAL COMPOSITION OF MARINE ALGAE[1]

By Margaret G. Macpherson and E. Gordon Young

Abstract

Fourteen species of the commoner marine algae of Nova Scotia have been analyzed for their content of water, nitrogen, ash, calcium, phosphorus, and iron. These algae are remarkable for their consistently high ash content. The minimum value was 12.3% in *Ahnfeltia plicata* and the maximum 45.4% in *Enteromorpha intestinalis*. The average content was 24.9%. Differences in composition have been observed between the green, red, and brown classes of algae.

It has been estimated that the plants of the sea equal in quantity the plant growth on land. Be that as it may, they have been used by man scarcely at all and may be regarded as a valuable potential natural resource. Our knowledge of the chemical composition of seaweeds, however, is very fragmentary. For Canadian waters of the Atlantic coast there are only the analyses of Butler (3, 4), mainly for six of the commonest species and with particular reference to potassium and iodine.

Within the past 10 years the commercial importance of certain species has become apparent,—notably *Chondrus crispus* (Irish moss) for the production of carrageenin and species of the *Laminariales* (kelp) and *Fucales* (rockweed) for the extraction of alginates. Our ignorance of the general composition of marine algae stimulated this initial survey. It has been directed towards those constituents of nutritional importance because of the increased use of algal extracts in the food industry. Only the commoner species to be found on the coast of Nova Scotia have been analyzed and the selection was based on the survey of Bell and MacFarlane (1).

Experimental

Preparation of Material

The plants were transported in sea water from the source to the laboratory. Identification of species was based on the manual of Taylor (16). Each analysis in this study represents a mixed sample prepared from a number of plants collected from the same place at the same time.

The determination of moisture was done on plants from which the adhering water was removed with cheesecloth. Large plants were minced in a food chopper. The error introduced by this procedure is apparently small (3). The plants were dried for 30 to 48 hr. in an oven at 100° C. They were then ground in a Wiley mill to pass through a sieve of No. 20 mesh. Prior to final weighing they were again dried in the oven and placed in a desiccator.

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Contribution from The Department of Biochemistry, Dalhousie University, Halifax, N.S., and The Nova Scotia Research Foundation.

Methods

Moisture determination was done on 10 to 40 gm. of the fresh seaweed by drying to constant weight at 100° C.

Total lipids were extracted from dried samples with ether for 16 hr. in a Soxhlet apparatus. The amount found was so small that only six species were analyzed.

The Kjeldahl–Gunning method was used for the determination of total nitrogen on moisture-free samples.

The ash content was found by igniting 2 to 4 gm. in an electric muffle furnace maintained at about 600° C. This required about eight hours.

Phosphorus was determined on approximately 1 gm. of the dried sample after digestion with 60% perchloric acid in a 50 ml. Kjeldahl flask according to the procedure of King (10). When digestion was complete the remaining liquid was made up to 100 ml. in a volumetric flask and a suitable aliquot (containing 0.2 to 0.8 mgm. of phosphorus) transferred to a 100 ml. volumetric flask. The color was developed according to the method of Fiske and Subbarow (6).

For the estimation of calcium, 15 ml. of the mixture after digestion with perchloric acid, referred to in the phosphorus determination, was pipetted into a 25 ml. volumetric flask. This was neutralized with 4 *N* ammonium hydroxide to about pH 6 as shown by bromcresol green. The calcium was then determined by the Clark–Collip modification (5) of the Kramer-Tisdall procedure.

Iron was estimated likewise on the perchloric acid digest by the method of Swank and Mellon (15) with sodium thioglycolate. Under these conditions the aliquot used did not require to be neutralized prior to the development of color.

The difference between the sum of the figures for protein ($N \times 6.25$), lipid, and ash and the total solids is usually assumed to be carbohydrate. Such values are only approximate because it is probably incorrect to calculate protein from the determinations of total nitrogen because of the presence of nonprotein nitrogen and the uncertainty of the level of nitrogen in the protein present (9, 13).

Results

The complete analyses are presented in Table I together with the site and time of collection. These plants are remarkable for the low level of total lipids that they contain, averaging about 2% in the species analyzed, and for the uniformly high level of ash content and total carbohydrate.

The content of moisture varied between 67 and 90% for all species examined. In two species it was not possible to carry out this determination because of the conditions of collection. The values recorded in Table I are in surprisingly good agreement with previous determinations by Butler (3). Our value for

TABLE I

COMPOSITION OF COMMONER SEAWEEDS OF THE MARITIME PROVINCES EXPRESSED AS PERCENTAGE ON A MOISTURE-FREE BASIS

Species	Moisture	*N*	Protein (*N* × 6.25)	Lipid	Ash	Calcium	Phosphorus	Iron	Class
Alaria esculenta (a)	82.4	2.13	13.3	—	29.2	1.45	0.43	0.026	Brown
Ahnfeltia plicata (b)	—	2.30	14.4	—	12.3	0.83	0.21	0.051	Red
Ascophyllum nodosum (c)	67.3	1.76	11.0	2.47	19.8	1.25	0.16	0.015	Brown
Chondrus crispus (d)	77.0	1.94	12.1	—	26.8	0.91	0.11	0.030	Red
Chordaria flagelliformis (e)	88.9	2.41	15.1	—	27.2	1.53	0.26	0.094	Brown
Dictyosiphon foeniculaceus (f)	88.2	2.56	16.0	—	23.1	2.02	0.24	0.053	Brown
Enteromorpha intestinalis (g)	—	2.58	16.1	—	45.4	0.68	0.21	0.582	Green
Fucus evanescens (c)	86.5	2.70	16.9	1.80	17.7	0.97	0.25	0.055	Brown
Fucus serratus (h)	74.9	0.59	3.7	—	14.5	1.19	0.13	0.044	Brown
Fucus vesiculosus (i)	76.6	1.73	10.8	0.43	21.5	2.93	0.18	0.067	Brown
Gigartina mamillosa (a)	76.2	3.62	22.6	—	21.2	0.51	0.29	0.036	Red
Halosaccion ramentaceum (j)	83.3	3.03	18.9	2.82	19.7	1.04	0.30	0.038	Red
Laminaria Agardhii (j)	88.8	2.09	13.1	1.96	27.2	1.09	0.22	0.015	Brown
Laminaria digitata (a)	86.5	1.69	10.6	—	27.9	1.29	0.23	0.018	Brown
Laminaria longicruris (f)	90.5	1.84	11.5	—	34.8	1.46	0.16	0.030	Brown
Rhodymenia palmata (c)	88.1	4.04	25.3	3.78	26.7	0.24	0.32	0.025	Red
Ulva Lactuca (a)	84.5	3.75	23.4	—	29.1	0.82	0.29	0.139	Green
Averages									
Phaeophyceae (10)	83.0	1.95	12.0	—	24.3	1.52	0.23	0.042	Brown
Rhodophyceae (5)	81.0	2.99	19.0	—	21.3	0.71	0.25	0.036	Red
Chlorophyceae (2)	—	3.17	20.0	—	37.3	0.75	0.25	0.360	Green
General average	83.0	2.40	15.0	2.21	24.9	1.19	0.24	0.078	

Site and time of collection

(a) Sandy Cove, N.S., August, 1947.
(b) Near West Pubnico, Yarmouth Co., N.S., August, 1948.
(c) Halifax, N.S., May, 1947.
(d) Offshore near Wedgeport, Digby Co., N.S., August, 1947.
(e) Bliss Island, near St. Andrews, N.B., July, 1947.
(f) St. Andrews, N.B., August, 1947.
(g) West Pubnico, Yarmouth Co., N.S., July, 1948.
(h) Pictou, N.S., August, 1947.
(i) Halifax, N.S., October, 1946.
(j) Halifax, N.S., June, 1947.

Laminaria longicruris is 91% as compared to 89; *Rhodymenia palmata*, 88 vs. 85; *Fucus vesiculosus*, 77 vs. 76; *Ascophyllum nodosum*, 67 vs. 70; *Gigartina mamillosa*, 76 vs. 68.

The variation in total lipids was 0.43 to 3.78% in six species and this can be compared with 0.04 to 1.73% as recorded by Reed (14) on air-dried material of Hawaii and 0.5 to 3% in the analyses of four species by Lunde (12). Haas and Hill (7) have observed an increase of total lipid with degree of emergence in different ecological zones.

The values for total nitrogen are close to those obtained by Butler (3). In most cases they are near the mode as listed by Lunde (12) except in the case of *Fucus serratus* where his values are 1.44 to 2.88% as compared with

0.59%. Values for six species are recorded for the first time. The mean for brown seaweeds is appreciably lower than those for red or green.

The variation in total ash content is 12.3 to 45.4% with an average of 24.9%. The highest value obtained was in the green alga, *Enteromorpha intestinalis*, and this suggests that there may be an unusual polysaccharide present in this species. The results also suggest a possible difference in this respect between the green marine algae and those of other classes. The agreement in various analyses for the same species in different parts of the world, collected at different times, is astonishingly good. This is shown in Table II for two species.

TABLE II

ASH VALUES AS PERCENTAGE OF DRY WEIGHT

	Ascophyllum nodosum	*Fucus vesiculosus*
Hendrick (8)	21.5	20.3
Butler (3)	22.2	20.7
Lunde (12)	21.0	22.0
Authors	19.8	21.5

The amount of calcium present in algae is appreciable and the analyses suggest a difference between the *Phaeophyceae* at 1.52% and the two other groups at 0.7%. Phosphorus is remarkably constant in all three classes at approximately 0.24%. Calculated from the mean values, calcium would be 4.8% of the ash content and phosphorus about 1%. There are very few such analyses in the literature. Lunde (12) found calcium at 2.95 and phosphorus at 0.18 as % of total solids in *F. vesiculosus* and calcium, 1.24 and phosphorus, 0.16% in *A. nodosum*. In the ash of the fronds of *Laminaria*, calcium constituted 3.5 to 6% and phosphorus, 2 to 4%. These figures are of the same order of magnitude as our own.

The content of iron appears to be much higher in green algae than in the other groups studied. The values obtained by Lunde (12) for *A. nodosum* and *F. vesiculosus* are identical with those in Table I. These appear to be the only analyses in the literature.

These analyses were performed on plants collected for the most part during the summer months. The work of Butler (4) on *Chondrus crispus*, of Lunde (11), and the recent extensive analyses of Black (2) on the *Laminariaceae* suggest important variations dependent on season and habitat. It is hoped to extend these analyses to establish our knowledge of these factors in relation to the species studied and to examine such constituents as proteins and carbohydrates in greater detail.

Acknowledgments

We wish to take this opportunity to express our grateful thanks to Prof. H. P. Bell for assistance in identification of the algal species, to Miss C. Sullivan for collecting samples at St. Andrews, N.B., and to the Nova Scotia Research Foundation for financial assistance that made this investigation possible.

References

1. Bell, H. P. and MacFarlane, C. The marine algae of the Maritime Provinces of Canada. Can. J. Research, 9 : 265-279. 1933.
2. Black, W. A. P. The seasonal variation in chemical constitution of some of the sublittoral seaweeds common to Scotland. J. Soc. Chem. Ind. (London), 67 : 165-168; 169-172; 172-176. 1948.
3. Butler, M. R. Comparison of the chemical composition of some marine algae. Plant Physiol. 6 : 295-305. 1931.
4. Butler, M. R. Seasonal variations in *Chondrus crispus*. Biochem. J. 30 : 1338-1344. 1936.
5. Clark, E. P. and Collip, J. B. A study of the Tisdall method for the determination of blood serum calcium with a suggested modification. J. Biol. Chem. 63 : 461-464. 1925.
6. Fiske, C. H. and Subbarow, Y. The colorimetric determination of phosphorus. J. Biol. Chem. 66 : 375-400. 1925.
7. Haas, P. and Hill, T. G. Observations on the metabolism of certain seaweeds. Ann. Botany, 47 : 55-67. 1933.
8. Hendrick, J. The value of seaweeds as raw materials for chemical industry. J. Soc. Chem. Ind. (London), 35 : 565-574. 1916.
9. Hoagland, D. R. Organic constituents of Pacific coast kelps. J. Agr. Research, 4 : 39-58. 1915.
10. King, E. J. The colorimetric determination of phosphorus. Biochem. J. 26 : 292-297. 1932.
11. Lunde, G. Seaweed as a source of (chemical) raw materials. Angew. Chem. 50 : 731-734. 1937. (*From* Chem. Abstracts, 31 : 8747. 1937.)
12. Lunde, G. Utilization of seaweed and kelp as fodder. Skand. vet.-tid. bakt. patol. kött-och mjölkhygien, 26-62. 1940. (*From* Chem. Abstracts, 34 : 3393. 1940.)
13. Okuda, Y. and Nakayama, S. The quality of "Asakusanori." (*Porphyra laciniata.*) J. Coll. Agr. Imp. Univ. Tokyo, 5 : 339-340. 1916. (*From* Chem. Abstracts, 11 : 3066. 1917.)
14. Reed, M. The economic seaweeds of Hawaii and their food value. Ann. Rept. Hawaii Agr. Expt. Sta. 61-88. 1906.
15. Swank, H. W. and Mellon, M. G. Determination of iron with mercaptoacetic acid. Ind. Eng. Chem. Anal. Ed. 10 : 7-9. 1938.
16. Taylor, W. R. Marine algae of the northeastern coast of North America. The Univ. of Mich. Press. Ann Arbor, Mich. 1937.

A STUDY OF TROUT STREAMSIDE COVER IN LOGGED-OVER AND UNDISTURBED VIRGIN SPRUCE WOODS[1]

By R. G. H. Cormack[2]

Abstract

In the present study of trout streams in mountainous headwater regions, the term "streamside cover" includes a wide strip of forest vegetation on both sides of the stream, rather than a narrow fringe of plants at the water's edge. Undisturbed stream margins in virgin spruce woods are compared with those in adjacent areas recently logged over in relation to shade production, water conservation, and protection against stream-bank erosion. Heavy, indiscriminate cutting along the stream margins has removed almost all the good shade producing trees, eradicated by exposure and mechanical means the former water conserving ground vegetation, and caused serious damage to the stream itself by logging operations. As the result of this study, the prohibition of all cutting along wide strips on both sides of the trout stream is strongly recommended in order to maintain suitable environmental conditions for trout and to preserve aesthetic values of fishing areas.

Introduction

A botanical survey, sponsored by the Alberta Department of Lands and Mines, was undertaken during the summer of 1944. The purpose of the survey was to obtain information concerning the vegetation of undisturbed and disturbed forest areas, to analyze the information and to relate it to the problems of soil erosion, water conservation, and trout stream management.

The region involved is a wide tract of mountainous country, designated as the Crowsnest Forest Reserve in southwest Alberta. On the Alberta side of the watershed all the valleys support dense stands of coniferous forest, either virgin or comparable well developed stands of second growth. The forest is of great importance as a protection for this part of the watershed in that it contains the headwaters of many important rivers. For a long time this well watered, forested section of the watershed has been known as one of the most important trout fishing regions in western Canada. However, within recent years there has been a reported decline in the trout populations. This observation, together with reports of the drying up of springs and of periodic floods and drought in the larger streams, indicates a common cause; the disturbance of the forest cover.

Foresters are well aware of the benefits derived from the study of virgin stands. Lutz (18) believes that a virgin stand offers the best place to investigate natural processes as they relate to tree growth and advises foresters to go to the virgin stand for knowledge of forest ecology on which to base their silvicultural practice. Hough (13) is also of the same opinion and states that a study of the climax virgin forest will show the tree species and kind of forest organism capable of using the site to best advantage.

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In regard to the present survey it was thought that a study of trout stream conditions in a virgin forest would be valuable not only in obtaining a fuller understanding of the changes produced by the disturbance of the forest cover but also in formulating a wise policy of trout stream protection and management. In a study of this kind not only is it essential to know the identity, abundance, and distribution of streamside plants but enquiry should also be made into their capacity to give shade and protection, to conserve soil and moisture, and to harbor food organisms. To attempt to satisfy all the above conditions, unassisted, in a rough terrain, and within a period of two months, was obviously out of the question. Under the circumstances, collecting was mainly restricted to virgin stands along the river valleys. Data in the form of field notes were obtained on the character of the streamside vegetation and forest soil, stream banks, and channels in undisturbed areas and in those areas disturbed by fire, logging, and grazing.

The present paper deals with observations made in only one of the river valleys surveyed, namely that of the Carbondale River. It records the streamside vegetation of undisturbed virgin stands and discusses the changes produced within these stands by logging operations. This paper lays no claim to represent a finished piece of work, but it is hoped that the final result will be a fuller understanding of the influence of the forest cover on the control of stream flow and on the welfare of trout. Trout stream management will be most successful when biologists understand nature's methods and apply them in maintaining a streamside cover best suited to the climate and soil of a given area.

Location and Physiography

The Crowsnest Forest covers an area of approximately 450 square miles in the southwest corner of the Province (Fig. 1). It takes its name from close proximity to the Crowsnest Pass, one of the most famous of Rocky Mountain Passes, and the route followed by the Crowsnest line of the Canadian Pacific Railway. At its widest point the reserve extends eastward from the provincial boundary a distance of about 20 miles and southward from the Crowsnest Pass to a point about 12 miles north of the International boundary.

The greater part of the reserve is very rugged and uneven, since it includes a portion of the Flathead Range of mountains. With the exception of a narrow range of foothills the transition from mountains to plains on the Alberta side of the watershed is very abrupt. The geology of this area according to Hume (15) is mainly of Precambrian strata.

The region is well watered and is drained by the Oldman River, tributary to the South Saskatchewan River system. Traversing the reserve and flowing eastward and north eastward into the Oldman River are the Carbondale River and a westerly branch of the Castle River. However, as mentioned previously this paper deals only with that part of the survey conducted in the Carbondale River valley.

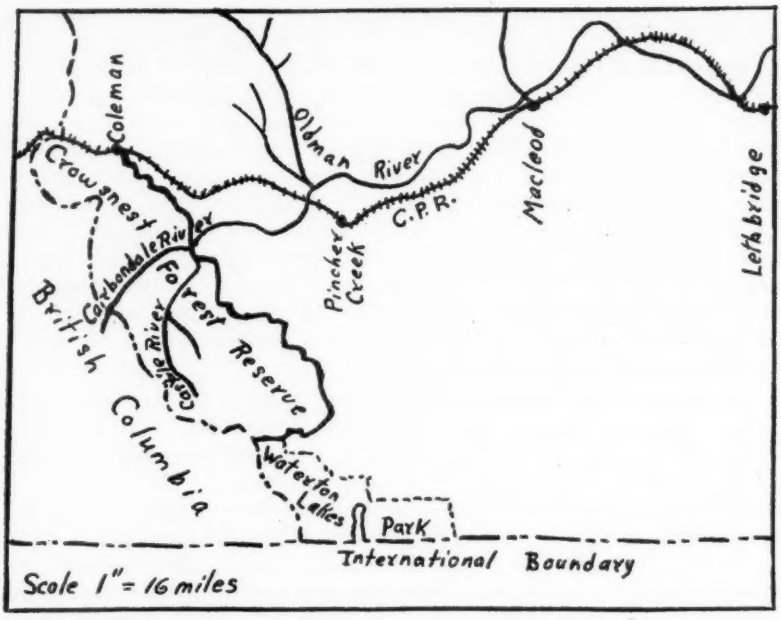

FIG. 1. *Map of southwestern Alberta showing location of the Crowsnest Forest Reserve and the Carbondale River.*

As shown in Fig. 2 the Carbondale River originates as a small spring-fed mountain stream near the Provincial Boundary and flows eastward through a narrow gap in the mountains designated by Cautley *et al.* (2) as North Kootenay Pass. According to the same authority the "Kootanie Indians" formerly crossed the mountains by this pass each summer to secure a supply of buffalo meat on the Alberta Plains. To-day because of the steepness of the pass particularly from the British Columbia side, this ancient travel route is merely a faint pack-trail much grown over and littered with windfall. On the Alberta side, the pack-trail, which keeps to the north side of the stream, descends open slopes and through dense forest to join a recently constructed road at a point where the latter crosses the Carbondale River. The pass itself is bounded on the north by the southern slopes of Kootenay Mountain (7930 ft.), and on the south by the northern slopes of Mt. McCarty (7748 ft.). Westward, on each side of the pass the mountain slopes are precipitous forming a narrow defile; eastward, the pass opens out and more gentle slopes become apparent. The ridges are separated by wide densely timbered valleys where springs and small tributary streams collect the runoff and flow to MacDonald, Gardiner, and Lost Creeks, which in turn empty into the Carbondale River.

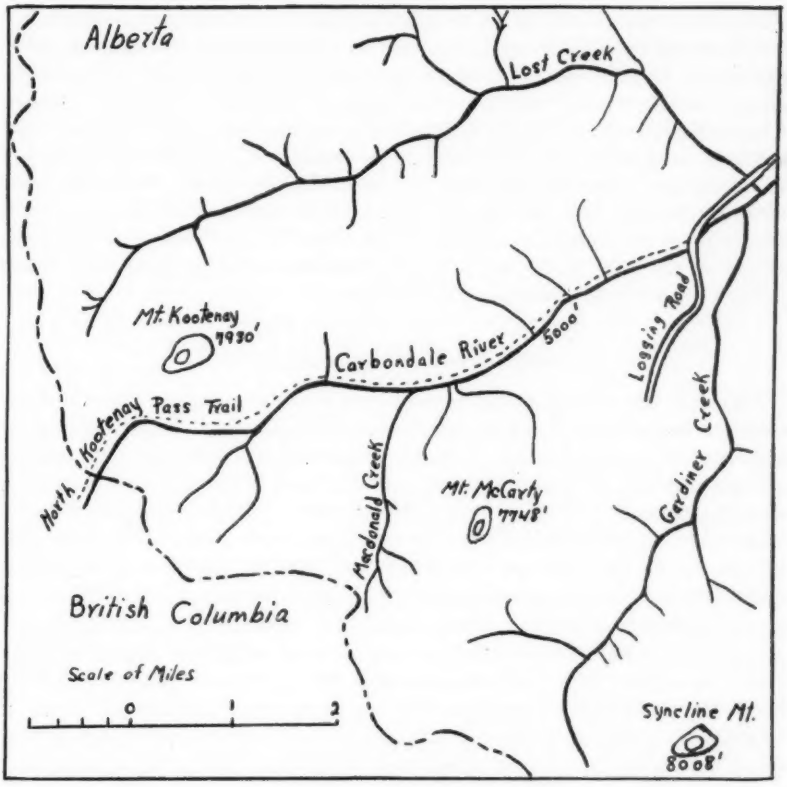

FIG. 2. *Map of the study area in the Carbondale River valley.*

The area selected for special study included that part of the river valley extending southwest along the river for a distance of about four miles above the mouth of Gardiner Creek, and north and south of here to the valleys of Lost and Gardiner Creeks, respectively (Fig. 2). Although referred to as creeks these two streams are of much the same size as the Carbondale River. Gardiner Creek, which originates at the base of Mt. Syncline (8008 ft.) formed the eastern boundary of the study area. A portion of Gardiner Creek valley was burned in 1936, while Lost Creek Valley is for the most part undisturbed.

Virgin coniferous forest occupies the greater part of the study area and extends west to the British Columbia Border. Spruce is the chief tree on the immediate banks of the river, wide river flats, and the lower level of adjacent slopes. That the species is white spruce (*Picea glauca*) seems most likely, though Engelmann spruce (*Picea engelmanni* (Parry) Engelm.) has been recorded for the same region. Considerable variation in the character of the cones suggests that hybridization occurs between these two species.

The general appearance of such stands is that of a closed, even aged forest characterized by a thick moss cover and by a shrubby and herbaceous undergrowth of variable density and composition. In this regard, these virgin spruce stands closely resemble the northern coniferous forest of eastern Canada described by Nichols (23) as being monotonously uniform over vast stretches of country and impressive in its simplicity. At the eastern end of the study area, aspen poplar (*Populus tremuloides*) occurs as a fringe bordering meadows or with lodgepole pine (*Pinus contorta* var. *latifolia*) in open mixed woods along the south-facing slope of the river. At the western end near the source of the river, lodgepole pine predominates while limber pine (*Pinus flexilis*) occurs on exposed slopes and ridges.

Undisturbed Streamside Cover

Although the term streamside cover is of common usage no definite statement as to the amount of marginal vegetation involved is to be found in the literature. As the present survey progressed it became evident that a greater portion of the forest vegetation was involved than merely a narrow fringe of plants at the water's edge. Consequently a strip of vegetation approximately 50 ft. in width on each side of the river was arbitrarily decided upon as more truly representing that part of the forest cover effective in providing streamside shade and protection. In typical spruce stands the character of the vegetation within such a narrow strip of ground is strikingly uniform with the exception of minor and irregular differences associated with rocky ledges, springs, and openings in the forest canopy caused by windthrown trees.

A list of plants recorded for undisturbed river margins is presented in Table II. A number of species have been omitted because they were either not mature at the time the survey was made or accurate determinations are not available. However, even if not complete, the list includes most of the characteristic species and certainly those most frequently encountered.

As already mentioned the virgin forest is essentially white spruce, very dense and containing trees of large size and good merchantable value. The largest spruce tree had a D.B.H. of 40 in. while the majority measured from 18 to 30 in., and ranged in height from 100 to 130 ft. In adjacent logged areas the stumps of this species commonly show ages of 200 to 250 years. After spruce, mountain fir is the most common species in the tall tree class. The tallest trees had a height of approximately 90 ft. and had an average D.B.H. of 18 in. Most of the tall fir trees are diseased and such firs constitute the majority of recent windthrown trees on the forest floor. In general seedling reproduction of spruce and fir is poor but reaches its best development under openings in the forest canopy due to the windthrow of overmature trees. Here large numbers of spruce and fir seedlings may be found growing on almost every moss-covered windfall.

Lodgepole pine is frequently present in the streamside coniferous cover but only as a minor constituent and since there is complete lack of reproduction of this species it is clear that lodgepole pine is passing out of the spruce dominated stands. Representatives of this species now in the stands, overshaded by the spruce, are in a dying condition. That balsam poplar is also disappearing is evidenced by the presence of very few individuals in the tall tree class and none at all in the small tree class. Overtopped trees of this species are common. Douglas fir is of such rare occurrence that it constitutes an unimportant element of the streamside cover. Representative trees are usually taller than the spruce and are in a healthy condition. Two tree species of extremely rare occurrence have been identified as the western cedar and ponderosa pine. These two species are native to British Columbia and have not been listed previously for this part of the Rocky Mountains Forest Reserve (8, 10).

In general, small trees of the leading tree species are few in number and well defined layers or strata of woody plants are poorly developed. Smaller spruce and mountain fir occur as scattered trees of apparently very slow growth in those places where they form part or all of the tall tree canopy. On the immediate banks, often overhanging the water, willows and alders, together with a number of other small trees and shrubs, fill in the intervening spaces between the tall spruce.

The shade provided by this kind of forest cover combined with the usually large amount of soil moisture produces a cool moist atmosphere that is most impressive, and is in decided contrast to adjacent cutover areas. For instance, at midday during the month of July there was an average difference of 33° F. between the atmospheric temperature of an undisturbed virgin stand and one recently denuded of all tall trees. In addition to reducing the rate of evaporation from the forest floor the shade produced by the tall trees is the chief factor determining the floristic composition and density of the ground vegetation.

In undisturbed spruce stream margins, the vegetation on the forest floor is a well developed layer whose composition and character is determined by shade, topography, and proximity to water.

The most characteristic and constant feature of the undisturbed virgin spruce woods is the luxuriant development of mosses, which form a continuous green carpet over the soil, boulders, and windfalls, and at the water's edge over stones, old logs, and projecting tree roots. The luxuriance of this moss carpet some 4 to 6 in. in depth, to those who have never observed it nor walked upon it seems almost unbelievable. The mosses decrease the rate of evaporation from the forest floor; through decay they form more humus and by retarding and absorbing rain and snow they promote the slow seepage of water to the soil beneath. Not only do they soak up water rapidly like a sponge but in the shade of the tall trees they hold it tenaciously even over long periods of dry weather. Along the overhanging banks of the river, springs, and small feeder streams, the mosses check the rapid runoff of surface water, thus preventing erosion and by sifting out silt and debris keep the

water clean and fresh. Typical samples of these mosses were shown on subsequent testing in the laboratory to have a water holding capacity of 300 to 600%. This figure as expressed in percentage represents the amount of water held by 10 gm. of air-dried moss.

The list of mosses (Table I), though by no means complete, includes the most common species and those that constitute the bulk of the moss carpet.

TABLE I

SPECIES FOUND ALONG UNDISTURBED STREAM MARGINS

(Symbols: A – abundant; C – common; O – occasional; R – rare)

Scientific name	Common name	Abundance
BRYOPHYTES	Mosses	
Cratoneuron filicinum (L.) Roth		C
Dicranum Bonjeani De Not.		C
Hylocomium splendens Hedw.		A
Hypnum Crista-castrensis (L.) Hedw.		A
Hypnum Lindbergii Mitt.		A
Hypnum Schreberi Willd.		A
Mnium affine Bland.		C
Mnium punctatum (L.) Hedw.		C
Polytrichum commune Hedw.		O
Polytrichum juniperinum Hedw.		O
Rhytidiadelphus Loreus (L., Hedw.) Warnst.		A
Rhytidiadelphus triquetrus (L., Hedw.) Warnst.		A
PTERIDOPHYTES		
Athyrium Filix-femina (L.) Roth	Lady fern	A
Cystopteris fragilis (L.) Bernh.	Fragile fern	C
Dryopteris disjuncta (Rupr.) Morton	Oak fern	C
Dryopteris spinulosa (L.) Ktze.	Toothed wood fern	C
Pteridium aquilinum var. *pubescens* (L.) Kuhn.	Bracken fern	R
Woodsia scopulina D.C. Eat.	Mountain cliff fern	O
Equisetum arvense L.	Common horsetail	A
Equisetum hyemale L. var. *robustum* (A. Br.) A.A. Eat.	Scouring rush	O
Equisetum pratense Ehrh.	Thicket horsetail	O
Equisetum scirpoides Michx.	Rush horsetail	A
Equisetum variegatum Schleich.	Variegated horsetail	O
Lycopodium annotinum L.	Bristly club moss	A
Lycopodium complanatum L.	Trailing club moss	C
SPERMATOPHYTES		
(Trees)		
Abies lasiocarpa (Hook.) Nutt.	Mountain fir	C
Acer Douglasii Hook.	Mountain maple	O
Alnus incana (L.) Moench.	Speckled alder	C
Alnus sinuata (Regel.) Rydb.	Alder	C
Picea glauca Voss	White spruce	A
Pinus contorta Loud. var. *latifolia* S. Wats.	Lodgepole pine	O
Pinus flexilis James	Limber pine	R
Pinus ponderosa Dougl.	Ponderosa pine	R
Populus trichocarpa T. & G.	Western balsam poplar	O
Populus tremuloides Michx.	Aspen poplar	R
Pseudotsuga taxifolia (Poir.) Rehder	Douglas fir	O
Salix glauca L. var. *glabrescens* (And.) Schn.	Blue-gray willow	C
Salix subcoerulea Piper	Blue-green willow	C
Sorbus scopulina Greene	Mountain ash	O
Thuja plicata D. Don	Western cedar	R

TABLE I—*Continued*

SPECIES FOUND ALONG UNDISTURBED STREAM MARGINS—*Continued*

(Symbols: A – abundant; C – common; O – occasional; R – rare)

Scientific name	Common name	Abundance
SPERMATOPHYTES—*Continued*		
(Shrubs)		
Amelanchier alnifolia Nutt.	Saskatoon	O
Cornus stolonifera (Michx.) Rydb.	Red osier dogwood	C
Lonicera involucrata (Richards.) Banks	Swamp honeysuckle	C
Menziesia glabella A. Gray	Menziesia	O
Pachystima Myrsinites (Pursh) Raf.	Mountain lover	O
Rhododendron albiflorum (Hook.) Rydb.	Mountain rhododendron	O
Ribes lacustre (Pers.) Poir.	Swamp black currant	O
Rubus idaeus L. var. *canadensis* Rich.	Wild red raspberry	O
Rubus parviflorus Nutt.	Salmonberry	R
Sambucus melanocarpa A. Gray	Elderberry	O
Shepherdia canadensis (L.) Nutt.	Buffaloberry	O
Spiraea lucida Dougl.	Spiraea	R
Symphoricarpos sp.	Snowberry	R
Vaccinium caespitosum Michx.	Dwarf bilberry	O
Vaccinium membranaceum Dougl.	Bilberry	O
Vaccinium oreophilum Rydb.	Huckleberry	R
Vaccinium scoparium Leiburg	Gooseberry	R
Viburnum eradiatum (Oakes) House	Mooseberry	O
(Sedges, rushes, and grasses)		
Carex atrosquama Mack.	Black-scaled sedge	C
Carex aurea Nutt.	Golden sedge	C
Carex Backii Boott.	Back's sedge	O
Carex Deweyana Schwein.	Dewey's sedge	C
Carex disperma Dewey	Soft-leaved sedge	C
Carex flava L.	Yellow sedge	C
Carex interior Bailey	Inland sedge	C
Carex lenticularis Michx.	Lenticulate sedge	O
Carex Mertensii Prescott	Merton's sedge	C
Carex Rossii Boott.	Ross's sedge	O
Carex rostrata Stokes	Rostrated sedge	C
Juncus balticus var. *montanus* Engelm.	Baltic rush	C
Juncus longistylis Torr.	Long-styled rush	C
Juncus Mertensianus Bong.	Slender-stemmed rush	O
Juncus saximontanus A. Nels.	Rocky mountain rush	C
Luzula campestris D.C. var. *multiflora* (Ehrh.) Celak.	Many-flowered wood rush	C
Luzula parviflora (Ehrh.) Desv.	Small-flowered wood rush	C
Agrostis exarata Trin.	Spike redtop grass	R
Calamagrostis canadensis (Michx.) Beauv.	Marsh reed grass	O
Cinna latifolia (Trev.) Griseb.	Wood reed grass	O
Elymus glaucus Buckley	Smooth wild rye	O
Glyceria (?) *elata* (Nash) Hitch.	Slender wood grass	O
Glyceria pauciflora Presl.	Manna grass	O
Melica spectabilis Scribn.	Purple onion grass	O
Melica subulata (Griseb.) Scribn.	Melic grass	O
Oryzopsis asperifolia Michx.	Mountain rice	O
(Herbaceous plants)		
Actaea spp.	Baneberry	C
Angelica Dawsoni S. Wats.	Angelica	O
Aquilegia flavescens S. Wats.	Mountain columbine	O
Arnica cordifolia Hook.	Heart-leaved Arnica	C
Aster spp.	Aster	O
Calypso bulbosa (L.) Oakes	Calypso orchid	R
Cardamine pennsylvanica Muhl.	Bitter cress	O
Castilleja mineata Benth.	Indian paint brush	R

TABLE I—*Concluded*

SPECIES FOUND ALONG UNDISTURBED STREAM MARGINS—*Concluded*

(Symbols: A – abundant; C – common; O – occasional; R – rare)

Scientific name	Common name	Abundance
SPERMATOPHYTES—*concluded*		
(Herbaceous plants)—*concluded*		
Chimaphila occidentalis Rydb.	Pipsissewa	R
Clintonia uniflora (Schult.) Kunth.	One-flowered Clintonia	A
Corallorrhiza trifida Chatelain	Early coralroot orchid	R
Cornus canadenis L.	Bunchberry	A
Disporum trachycarpum S. Wats.	Disporum	C
Epilobium angustifolium L.	Fireweed	O
Epilobium spp.	Fireweed	O
Erigeron salsuginosus (Richards.) A. Gray	Fleabane	O
Fragaria glauca (S. Wats.) Rydb.	Wild strawberry	O
Galium triflorum Michx.	Sweet scented bedstraw	O
Geranium Richardsonii Fisch. & Trautv.	White-flowered geranium	R
Geum sp.	Avens	R
Glycosma occidentalis Nutt.	Glycosma	R
Goodyera decipiens Hubbard	Rattlesnake plantain orchid	R
Habenaria dilatata (Pursh.) Hook.	Tall leafy white orchid	C
Habenaria obtusata (Pursh.) Richards.	Blunt-leaf orchid	O
Habenaria saccata Greene	Green bog orchid	C
Heracleum lanatum Michx.	Cow parsnip	O
Heuchera cylindrica var. *septentrionalis* R.B.L.	Alumroot	R
Lathyrus ochroleucus Hook.	Cream-colored vetchling	O
Linnaea americana Forbes	Twinflower	A
Listera convallarioides (Sw.) Torr.	Twayblade orchid	R
Listera cordata (L.) R. Br.	Twayblade orchid	R
Listera caurina Piper	Twayblade orchid	R
Mimulus guttatus DC.	Yellow monkey flower	R
Mimulus Lewisii Pursh.	Crimson monkey flower	R
Mitella nuda L.	Bishop's cap	C
Mitella pentandra Hook.	Bishop's cap	O
Moneses uniflora (L.) A. Gray	One-flowered wintergreen	O
Osmorrhiza obtusa (Coult. and Rose) Fernald	Sweet cicely	C
Parnassia fimbriata Konig.	Grass of Parnassus	O
Pedicularis bracteosa Benth.	Lousewort	C
Phaca americana (Hook.) Rydb.	Arctic milk vetch	R
Pyrola asarifolia Michx.	Liver-leaf wintergreen	C
Pyrola bracteata Hook.	Purple-flowered wintergreen	R
Pyrola chlorantha Swartz.	Greenish-flowered winter-green	C
Pyrola minor L.	Small-flowered wintergreen	R
Pyrola secunda L.	One-sided wintergreen	O
Ranunculus Bongardi Greene	Wood buttercup	O
Saxifraga arguta D. Don.	Saxifrage	O
Saxifraga Mertensiana Bong.	Saxifrage	O
Senecio triangularis Hook.	Brook ragwort	C
Smilacina racemosa (L.) Desf.	False spikenard	C
Smilacina stellata (L.) Desf.	Star-flowered Solomon's seal	C
Stenanthium occidentalis A. Gray	Stenanthium	C
Streptopus amplexifolius (L.) DC.	Twisted stalk	A
Thalictrum megacarpum Torr.	Meadow rue	C
Tiarella unifoliata Hook.	False miterwort	C
Trollius albiflorus (A. Gray) Rydb.	Globeflower	O
Urtica gracilis Ait.	Slender nettle	O
Veratrum Eschscholtzianum (R. & S.) Rydb.	False hellebore	C
Veronica americana Schwein.	American brooklime	C
Viola glabella Nutt.	Yellow-flowered violet	A
Viola nephrophylla Greene	Northern bog violet	A
Zygadenus elegans Pursh.	White camass	O

Closely associated with the mosses are lichens and liverworts. The chief and most abundant lichen is *Peltigera aphthosa* (L.) Willd., while many others including various species of the so-called reindeer moss (*Cladonia* spp.) are common on moss-covered windfalls and on the trunks of trees.

Windfalls form a constant and characteristic part of the mossy ground cover in all undisturbed river margins. Partly decayed when the trees fall, windfalls decompose further on the ground and within a short time they become covered by a thick mat of mosses, liverworts, and lichens. In general, throughout the stand and particularly on gentle slopes leading down to the river's edge, they aid greatly in checking the runoff of rain and melting snow. Decaying, punky windfall material has a water-holding capacity of 435%. In one instance windfalls of this kind were still wet after a dry period of 20 days during the month of July.

Closely associated with the moss vegetation, from which it is partly derived, is the forest soil. Beneath the mosses as seen in profile is a well defined, black organic layer consisting of partially or wholly decayed vegetable matter.

By virtue of the humus the organic layer possesses a loose, porous texture, which increases permeability and enables it to absorb large quantities of rain and to conduct the water downwards, and into small springs. In undisturbed spruce woods the organic layer varies in thickness from $1\frac{1}{2}$ to $2\frac{1}{2}$ in. and has a water-holding capacity of 310%. In sharp contrast to the organic layer, the underlying light colored mineral soil is usually claylike in texture and is frequently securely packed. It has a water-holding capacity of 48% and when the protective moss and organic covering is removed it soon becomes baked hard in the sun and almost impervious to water.

In general the undergrowth of shrubby and herbaceous plants is poorly developed (Table I). As mentioned already there is a close relationship between the density of the undergrowth and the amount of direct sunlight reaching the forest floor. Where the tree canopy is completely closed it is either nonexistent or very thin, but improves as the sunlight increases towards the river's edge, along game trails, and in openings in the tree canopy caused by wind-thrown trees.

On the whole, pronounced grouping or dominance of single species, with the exception of localized communities, is not evident; instead differences in the frequency of plant groups is more apparent. For instance in typical spruce stands the thick moss carpet is characterized by a thin scattering of shrubs, ferns, horsetails, club mosses, and a small but fairly constant number of herbaceous flowering plants. In the vicinity of springs, and in and around wet boggy ground, ferns, horsetails, and sedges are most plentiful, accompanied by orchids and other rare plants. Grasses are absent except along game trails and localized open areas. Near the river margin the mossy overhanging banks are characterized by a growth of shrubs, ferns, horsetails, sedges, and various shade-loving flowering plants. Along the more densely shaded parts of the river these same kinds of plants extend down to the water

where together with a few scattered grasses they constitute a thin interrupted fringe among moss-covered stones and partly submerged windfalls. Where the river widens, particularly at a bend, the shrubby and herbaceous cover reaches its best development and includes a number of flowering plants quite commonly found in open spaces.

Although all the plants listed in Table I are characteristic, the herbaceous species, which together with the mosses, ferns, horsetails, club mosses, and sedges appear to stand out most clearly as indicating the moist cool environment of spruce streamside margins, are: *Streptopus amplexifolius*, *Stenanthium occidentalis*, *Clintonia uniflora*, *Cornus canadensis*, *Linnaea americana*, all the orchids and wintergreens. Certainly these plants are the first to wither and perish from lack of shade on the removal of the tall trees.

The River

The Carbondale River as it flows through the undisturbed virgin forest is a typical meandering mountain stream. In the study area the river varies in width from 15 to 25 ft. and in midstream from 1 to 2 ft. in depth. With the exception of a few old established beaver ponds the water flows swiftly over a clean stony, gravelly bottom completely free from green scum (green algae) and water weeds (Fig. 3). Under the protective tree cover a luxuriant growth of mosses, ferns, horsetails, and shade-loving flowering plants cover the overhanging river margins and spread downward over the stones and windfalls to the water's edge (Fig. 4). Springs and small feeder streams run crystal clear over clean gravelly beds, in narrow deep-seated channels often covered by mosses, ferns, windfalls, and protecting tree roots. In this way water that seeps slowly into springs and creeks reaches the river with the minimum of loss.

In this stream, shade is always present, though in varying degrees of density. The amount of variation is due to differences in topography, to differences in the grouping and spacing of both trees and shrubs, and again to the presence of gaps in the forest canopy resulting from the windthrow of overmature trees.

Shade affects trout indirectly by keeping the temperature of the water cool. Available information on trout stream management indicates that temperature is the most important factor determining the distribution of trout (11, 14, 25). The maximum temperature for natural self-sustaining brook trout waters has been set at about 66° F. When the temperature goes higher than this for any considerable period of time the trout disappear. Those streams containing water at the maximum temperature for trout are more favorable than very cold waters where the food supply is limited. Lower temperatures favor spawning but where the temperature of the stream remains low at all times the trout population may become resident and of slow growth. It has also been noted that brook trout are less abundant and usually disappear where they have numerous summer associates (minnows, suckers, etc.). These associates are added in a regular order, the increase in numbers being correlated directly with a rise in the temperature of the water.

FIG. 3. *The Carbondale River in undisturbed virgin spruce woods.*

FIG. 4. *Shady river margin shown in Fig. 3 with tall spruce on the immediate bank, alders and willows overhanging the river, and a thick continuous carpet of absorbent mosses extending over the ground to the water's edge.*

In the present survey a number of temperature readings shows that the water temperature of the Carbondale River satisfies the conditions necessary for the good growth of trout. During the month of July, the average daily water temperature in undisturbed spruce stands was 47° F. and in beaver ponds under the same type of cover 53° F. The study did not include adjacent, less densely wooded areas and open beaver meadows, which, until logging operations began in this region, were easily accessible to trout. From the data obtained along the Carbondale River, the height of the streamside cover was found to be an important factor for the production of streamside shade. In the writer's opinion the growth of a few tall spruce near the water's edge is much more effective in this regard than a dense low growth of willows, alders, and shrubs. As the survey progressed it became increasingly clear that the arrangement of tall trees, small trees, and shrubs as they grow in undisturbed virgin forest is the best combination in producing a variable amount of streamside shade and in keeping the temperature of the water below the maximum tolerated by trout. The occurrence of a fair number of Rocky Mountain whitefish and trout in this part of the river strengthened the validity of this opinion.

A study of the relationship of the forest streamside cover to the kind and abundance of trout food did not lie within the scope of the present survey. Although studies in this field are still in the pioneer stage it is known that a considerable amount of trout food, mainly insects, comes from the land (12, 20, 21, 22). In a recent study of Wisconsin trout streams, Anderson (1) concludes that trout will select a given area more from the standpoint of food than of cover, although these two factors may go hand-in-hand.

Logged Streamside Cover

The cutting of a portion of the virgin forest under investigation provided an excellent opportunity to compare and to contrast conditions in both uncut and cut-over virgin stands in the same region.

The cutting of mature timber, mainly white spruce, but also some Douglas fir and lodgepole pine in the Carbondale River valley began in 1943 and was still in progress during the time of the survey. By the beginning of the spring of 1944, the whole width of the river flat extending for a distance of about one-half mile east of Gardiner Creek road, and about one-quarter mile west had been cut, as well as a wide area extending south of the river for nearly a mile (Fig. 2). During early spring of 1944 logging operations were continued on both sides of the road and by the month of July had extended both south and southwest toward the area of Macdonald Creek.

It is well known that the cutting of mature trees alters the natural forest conditions and that no matter how carefully the work is done there is always a certain amount of damage to the remaining vegetation. According to Dwight (8) ordinary logging in spruce stands nearly always opens up the stand too much, leaving only scattered seed trees, with the result that the original

moss cover dries out and the growth of grass and other unfavorable plants is encouraged. Heavy logging as exemplified in the Carbondale River valley brings about still more marked changes along the lines indicated above for ordinary logging. Here, because timber berths sold under old Dominion licenses allowed for clear cutting to a 7-in. diameter, all the good spruce and Douglas fir trees have been cut, leaving uncut the injured, deformed, poor grade trees, and species of low merchantable value, mainly fir and pine. However, if not typical for the whole reserve, the cutting of timber in this area is a well defined example of the maximum amount of damage that can be caused to the streamside cover of a first-class trout stream by heavy, indiscriminate cutting.

The most obvious change is the drying out and subsequent death and disappearance of the mosses, ferns, horsetails, club mosses, and shade-loving flowering plants. In some areas the mosses and the layer of black surface soil, in drying out, have separated from the yellow claylike subsoil and although the surface layers still soak up water during rain they lose it soon afterwards by evaporation. In other areas the surface layers have been mechanically stripped from the subsoil by the action of bulldozers, while in still others they have been obliterated under piles of debris, slashings, and uprooted trees. This accumulation of rubbish, together with piles of sawdust, constitute a serious threat of future fires. With the removal of the mossy ground cover and black surface soil the exposed subsoil becomes baked hard in the sun, and water, which formerly seeped slowly underground, now either runs off rapidly or collects in stagnant pools and evaporates.

The changes produced along the margins of the Carbondale River can best be described with reference to Figs. 5, 6, 7, and 8. Throughout the logged-over area every sound tree has been cut at the water's edge. Those that remain are poor grade trees and many of these with their roots damaged have since blown down or are in a dying condition. Replacing the former community of mosses and associated shade-loving plants, are species formerly less abundant or completely excluded (Table II). Plants in the first category that appear to thrive better under conditions of increased sunlight include some of the small trees and shrubs and a number of the hardier herbaceous species. New plants that are progressively coming in either from adjacent aspen woods and meadows or being introduced as a result of logging operations are mainly weeds and coarse grasses. Thus formerly forested streamsides are gradually changing to a wilderness of willows and weeds. This new type of streamside cover produces scarcely any shade and because it is much less effective in retarding the runoff of surface water, streambank erosion is well advanced. In this area the construction of logging roads has accelerated soil erosion, causing heavy silting of the river channel and of the water itself. Logging roads and sunken causeways add to the destruction of the former mossy vegetation and greatly disturb the flow of springs and small feeder streams. Consequently much water no longer reaches the river but settles in shallow muddy pools and evaporates. Chamberlain (3) and Tarzwell as

FIG. 5. *The Carbondale River in a logged-over area.*

FIG. 6. *Exposed muddy river margin shown in Fig. 5, littered with debris and with the former water-conserving ground vegetation completely destroyed. Stream-bank erosion is well advanced.*

FIGS. 7 and 8. *The Carbondale River in logged-over area, showing removal of all sound shade-producing trees and the river channel blocked completely over long distances.*

TABLE II

SPECIES FOUND ALONG LOGGED-OVER STREAM MARGINS

Scientific name	Common name	Abundance
Agropyron repens (L.) Beauv.	Quackgrass	C*
Alnus incana (L.) Moench.	Speckled alder	C
Alnus sinuata (Regel.) Rydb.	Alder	C
Amaranthus sp.	Pigweed	O*
Amelanchier alnifolia Nutt.	Saskatoon	O
Arabis glabra (L.) Bernh.	Rock cress	O*
Arnica cordifolia Hook.	Arnica, heart-leafed	C
Aster spp.	Asters	A
Beckmannia syzigachne (Steud.) Fern.	Slough grass	O*
Bromus pumpellianus Scribn. etc.	Brome grass	A*
Calamagrostis canadensis (Michx.) Beauv.	Marsh reed grass	C
Calamagrostis inexpansa A. Gray	Northern reed grass	O*
Calamagrostis rubescens Buckl.	Pine grass	O*
Chenopodium album L.	Lambs'-quarters	C*
Cirsium arvense (L.) Scop. etc.	Canada thistle	C*
Cirsium spp.	Thistles	C*
Cornus stolonifera (Michx.) Rydb.	Red osier dogwood	A
Elymus glaucus Buckley	Smooth wild rye	C
Epilobium angustifolium L.	Fireweed	A
Erigeron spp.	Fleabanes	C*
Erysimum cheiranthoides L.	Wormseed mustard	C*
Fragaria glauca (S. Wats.) Rydb.	Wild strawberry	O
Geranium Bicknellii Britt.	Wild geranium	O*
Gilia gracilis Hook.	Gilia	O*
Heracleum lanatum Mich.	Cow parsnip	C
Osmorrhiza obtusa (Coult. & Rose) Fernald	Sweet cicely	C
Phleum pratense L.	Timothy	C*
Polygonum spp.	Knotweeds	C*
Populus spp.	Poplars	O
Prunella vulgaris L.	Selfheal	O*
Ribes lacustre (Pers.) Poir.	Swamp black currant	O
Rosa spp.	Wild roses	O*
Rubus idaeus L. var. *canadensis* Rich.	Wild red raspberry	C
Rubus parviflorus Nutt.	Salmonberry	C
Salix glauca L. var. *glabrescens* (And.) Schn.	Blue-gray willow	C
Salix subcoerulea Piper	Blue-green willow	C
Senecio pseudaureus Rydb.	Thin-leaved ragwort	C*
Senecio triangularis Hook.	Brook ragwort	C
Silene Menziezii Hook.	Catchfly	O*
Spiraea lucida Dougl.	White meadowsweet	C
Symphoricarpos sp.	Snowberry	C
Taraxacum officinale Weber	Dandelion	C*
Urtica spp.	Nettles	C

* *Introduced species.*

quoted by Davis (7) contend that the heavy silting of the water as the result of streambank and roadbank erosion decreases the trout-carrying capacity of all streams and may render the planting of new trout in some streams ineffective.

In this region no attempt has been made to safeguard the river. Slash, whole trees, and debris of all kinds have been piled or thrown into the water (Figs. 7 and 8). In many instances this practice has resulted in the clogging of the river for long distances and there is more than one example where the

river has been so thoroughly blocked that the water has been forced into a new channel. Indeed if it were done deliberately the destruction of the Carbondale River in the cut-over area could not be more complete.

Whether seedlings of spruce, fir, and lodgepole pine will be able to germinate and grow in the cut-over areas is a question that could not be settled at the time the survey was made. However, the widespread destruction of the surface soil, the almost complete absence of sound seed trees, and high soil temperatures indicate that natural reproduction of coniferous trees has been put back for a great many years.

Conservation Problems

Remote mountain and foothill streams have long been associated with good trout fishing in the minds of anglers. However, to-day, in many such streams, decreased stream volume and unstabilized flow of water are causing heavy losses of trout. With the realization that these deleterious changes in water supply are related to changes in the vegetative cover (5, 6, 9, 17, 24, 25, 27, 28) more attention is being given by experts in stream management to the study of conditions in the headwater regions. For instance, Tarzwell, as quoted by Davis (7), believes that stream improvement means environmental improvement. In order to have lasting stream improvement, in his opinion, it is necessary to get out on the watershed and remedy as far as possible the unfavorable conditions that have damaged the streams. Feast, as quoted by the same authority (7), is convinced from his experience in managing Colorado trout streams that any stream that has a good watershed is a good trout stream and any so-called improvement work upon it would prove to be a needless expense. Miller (19), in a recent article summarizing his observations of Canadian trout streams, states: "Effective watershed protection, restoration of vegetative cover along denuded headwaters and other stream improvement measures give more promise of better fishing than rearing fish in hatcheries and dumping them into unsuitable waters."

Evidence obtained in the present survey of the Carbondale River shows that the streamside cover in undisturbed virgin spruce stands has a twofold influence, first, as it influences the conservation of water as an integral part of the watershed protection forest, and second, as it influences the stream itself.

The advantages of this type of forest cover in controlling the flow of water, and the detrimental changes produced in this regard as the result of logging operations have been discussed briefly above. Although the importance of implementing sound forestry practices in the cutting of timber in valuable watershed areas cannot be overemphasized, a thorough discussion of these practices does not lie within the scope of the present paper. Suffice it to say here, that with the accumulation of knowledge on restoration and improvement of watersheds, foresters are placing more emphasis on the relation of forestry to water supplies. In this connection, Connaughton and Wilm (4) describe certain timber-cutting methods that achieve the double purpose of harvesting

a good crop of timber while enhancing stream flow. They point out however, that the practice of forest cutting to improve water supplies cannot be applied indiscriminately. "If floods and erosion offer no particular problem and regulation of flow is achieved by reservoirs, the cut should be frequent and as heavy as may be compatible with acceptable economic returns. If natural regulation of flow is more important than water-yield, cutting should be lighter and a fairly complete forest canopy should be maintained. If flood and erosion hazards are high, cutting must be held to a minimum—no more than enough to keep the forest healthy and productive." With regard to headwater streams such as the Carbondale River it would appear that natural stream flow is most desirable to maintain satisfactory stream conditions for trout. Consequently cutting should be conservative, intelligently managed, and closely supervised.

The particular advantages of undisturbed virgin stands in providing shade and protection to the stream itself have been dealt with above. However, the value of shade in promoting the luxuriant growth of absorbent mosses, and regulating water temperatures for trout is worthy of special consideration. For the production of streamside shade it is height of the vegetative cover that counts and the forest cover bordering the Carbondale River has this characteristic by virtue of the giant spruce. In certain places, particularly where the river is comparatively narrow, shade may be almost continuous. Dense, unbroken shade over the whole length of a trout stream according to Huntsman (16) may not be desirable from the standpoint of trout food, but in mountainous headwater regions this condition seldom arises. Taking the Carbondale River as an example, topographic differences and modification of the forest cover by fire and by the grazing of game animals since time immemorial all go to produce variable environmental conditions for trout with different amounts of shade. Before cutting began in this part of the Carbondale River valley, deep shaded pools, less densely shaded shallow riffles and eddys, stretches of open comparatively quiet water, and numerous tributaries were accessible to trout. Now, as the result of logging operations, streambank and road bank erosion has caused heavy silting of the former gravelly channel over long distances, while the clogging of the river at numerous points, and the loss of the protective cover has seriously interfered with the feeding and spawning habits of trout by greatly restricting their movements. Apart from the standpoint of shade and protection the value of the virgin forest streamside cover in maintaining the natural beauty and attractiveness of the river cannot be overemphasized and in this respect alone is greatly superior to the rank low growth of weeds, willows, and shrubs that invariably follows logging operations.

The cutting of timber is by no means the only cause of disturbance to the streamside cover in watershed areas, but since it comes directly under the jurisdiction of the watershed manager, suitable protective measures can be

devised and barring fire can be rigidly enforced. From evidence obtained in the present survey one measure of stream protection that seems most desirable would be the prohibition of all cutting along wide strips on both sides of the stream. There is considerable precedent for advising a policy of this kind, as multiple use forestry admits noncutting in certain areas, if it is genuinely needed (17, 26). The width of the strips to be left uncut will undoubtedly vary with the individual stream and with the type of forest cover. Taking conditions in the Carbondale River Valley as more or less general for this part of the watershed the writer suggests as a beginning point a strip of at least 60 ft. on each side of the stream. Certainly the uncut areas should be wide enough to provide the maximum of shade and protection to both stream and streamside cover and to preserve the natural attractiveness of the stream. Also they should be extensive enough to include the stream's source, springs, and small feeder tributaries. This protective strip will also provide a much needed supply of tree seeds. Consideration for the protection of the stream must be made well in advance of logging operations and whatever the optimum width of the protective border may be, the watershed manager will be obliged to include its attainment in his plans.

Confirmatory evidence that protective strips should be of a good width was obtained by a study of streamside conditions in the adjoining valley of the Castle River. In 1936 a fire destroyed the whole valley with the exception of strips of unburned trees bordering both sides of the river. Where the strips are extremely narrow some shade is still provided by the tall trees but because of the poor condition of the ground cover, streambank erosion has started and springs and small feeder streams have dried up. Where the unburned strips measure 60 to 100 ft. or more in width, conditions bordering the river are identical to those in undisturbed virgin spruce woods.

Although the stream must be protected against mechanical damage, overall stream protection can only be achieved by the wise management of the whole watershed. In short, if the water-holding capacity of more or less undisturbed forest areas are maintained at a high level and that of burned and cutover areas restored, there will be no cause for concern about the streams themselves nor about the welfare of trout.

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References

1. ANDERSON, H. A. Classification of trout streams. Wisconsin Conservation Bull. 8 : 21-23. 1943.
2. CAUTLEY, R. W., WALLACE, J. N., and WHEELER, A. O. Report of the commission appointed to delimit the boundary between the Provinces of Alberta and British Columbia. Part 1. 1-191. 1917.
3. CHAMBERLAIN, T. K. Research in stream management in the Pisgah National Forest. Trans. Am. Fisheries Soc. 72 : 150-176. 1942.
4. CONNAUGHTON, C. A. and WILM, H. G. Post-war management of western forested watershedlands for water-yield. Trans. Am. Geophysical Union. 1944.
5. CORMACK, R. G. H. Green forests for good fishing. Outdoor Canada. April, 1946.
6. COVENTRY, A. F. Desiccation in Southern Ontario. Trans. Roy. Soc. Can. V, 34 : 15-23. 1940.
7. DAVIS, H. S. The management of trout streams. Trans. Sixth North American Wildlife Conference. 169-179. 1941.
8. DWIGHT, T. W. Forest conditions in the Rocky Mountains Forest Reserve. Can. Dept. Interior, Forest Service. Bull. 33. Ottawa. 1913.
9. GRAHAM, E. H. Natural principles of land use. Oxford University Press, Toronto. 1944.
10. HALLIDAY, W. E. D. and BROWN, A. W. A. The distribution of some important forest trees in Canada. Ecology, 24 : 353-373. 1943.
11. HAZZARD, A. S. Low water temperature, a limiting factor in the successful production of trout in natural waters. Trans. Am. Fisheries Soc. 63 : 204-207. 1933.
12. HAZZARD, A. S. and MADSEN, M. J. Studies of the food of the cutthroat trout. Trans. Am. Fisheries Soc. 63 : 198-203. 1933.
13. HOUGH, A. F. A climax forest community on east Tionesta Creek in northwestern Pennsylvania. Ecology, 17 : 9-28. 1936.
14. HUBBS, CARL L., GREELEY, JOHN R., and TARZWELL, CLARENCE M. Methods for the improvement of Michigan trout streams. Inst. Fish Research. Bull. No. 1. 1932.
15. HUME, G. S. Can. Dept. Mines. Geol. Rept. No. 2329. Part B. Ottawa. 1933.
16. HUNTSMAN, A. G. Man's effect on Ontario streams and fish. Conservation in south central Ontario. pp. 67-74. The King's Printer, Toronto. 1948.
17. KITTREDGE, J. Forest influences. McGraw-Hill Book Co., New York. 1948.
18. LUTZ, H. J. The vegetation of Hearts' Content, a virgin forest in northwestern Pennsylvania. Ecology, 11 : 1-29. 1930.
19. MILLER, R. B. Position of the hatchery in fish culture. Outdoor Canada. May, 1946.
20. MOROFSKY, W. F. A preliminary survey of the insect fauna of some typical Michigan trout streams. J. Econ. Entomol. 28 (1) : 82-86. 1935.
21. MOROFSKY, W. F. Survey of insect fauna of some Michigan trout streams. J. Econ. Entomol. 29 (4) : 749-754. 1936.
22. MOROFSKY, W. F. A comparative study of the insect food of trout. J. Econ. Entomol. 33 (3) : 544-547. 1940.
23. NICHOLS, G. E. The hemlock-white pine—northern hardwood region of eastern north America. Ecology, 16 (3) : 403-422. 1935.
24. PEARSON, H. S. Little waters. Soil Conservation Service. 1936.
25. RICHARDSON, A. H. The Ganaraska watershed. Dominion and Ontario Governments. Toronto. 1944.
26. SHOW, S. B. Integrating recreation and esthetics into multiple purpose forest management. The Forestry News Digest. March, 1937.
27. ZOHN, R. Forests and water in the light of scientific investigation. U.S. Government Printing Office. Washington, D.C. 1927.
28. ZOHN, R., BARRETT, L. I., and HOYT, W. G. Michigan's water problems. Mich. Dept. Conservation. Lansing, Mich. 1944.

ELECTROPHORETIC PROPERTIES OF PEA PROTEINS[1]

By L. R. Wetter[2] and A. G. McCalla[3]

Abstract

The soluble proteins of pea meal were extracted with phosphate buffer and fractionally precipitated with ammonium sulphate. An electrophoretic analysis of each preparation was made, and the amount and mobility of each component determined. There are probably three distinct components in the pea protein and one of these at least is a protein system, which tends to separate into further components under some conditions. The components were not at all well separated by fractional precipitation, and the separation was decidedly affected by the method of preparation and the pH of the precipitation medium.

Introduction

Very little work on the nature of the proteins in peas has been carried on since Osborne and Harris (5) published the results of an investigation on fractionation. These and earlier (4) results indicated that there were three distinct proteins in peas, two of them, legumin and vicilin, being globulins, and the third, legumelin, being an albuminlike protein. As with most of Osborne's work, the criterion of distinction used was solubility of the fractions in salt solutions, and it was believed that the proteins prepared using fractional precipitation from sodium chloride solutions and from ammonium sulphate solutions were identical.

Results of a preliminary electrophoretic survey of several plant proteins suggested that an electrophoretic study of the proteins extracted from pea meal would be an effective way of checking Osborne's conclusions. Solubility of these proteins is high, and fractionation by progressive precipitation is readily carried out. It was therefore decided to study the electrostatic properties of the protein material from pea meal in both the unfractionated condition and as fractionated by Osborne and Harris.

Material and Methods

The peas used throughout this study were from a commercial sample of the variety Early Blue, grown in western Alberta.

The peas were ground in a Wiley mill and the meal obtained reground in a ball mill for six hours. The reground meal was extracted with ether in a Soxhlet apparatus for 48 hr. After drying, the extracted meal was sifted through a 12xx silk screen.

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Extraction of the meal was carried out using 0.2 *N* phosphate buffer of pH 7.8 for six hours. The murky extract was put through a Sharples supercentrifuge to remove solids and large aggregates. This clarified extract is referred to as the "unfractionated extract".

The first fraction was prepared by diluting this extract by the addition of 10 volumes of distilled water and adjusting the dilution to pH 5.2 with 0.1 *N* hydrochloric acid. The dilution was stored overnight in a refrigerator at 3° C., then centrifuged to remove the precipitate. The precipitate was taken up in 0.2 *N* phosphate buffer, and the solution then dialyzed against 1500 cc. of buffer for approximately four days. This preparation is designated Fraction A1.

The remaining solution was made 60% saturated with respect to ammonium sulphate, and adjusted to a pH of 5.2. The precipitate was removed by centrifuging, and prepared for electrophoretic determinations in a manner similar to that used with Fraction A1. This fraction is designated A2.

The solution remaining after precipitation of Fraction A2 was saturated with ammonium sulphate, and the above procedure repeated. The redissolved precipitate is designated Fraction A3.

A second extract was prepared and fractionation carried out omitting the dilution step. The clarified extract was made 60% saturated with respect to ammonium sulphate at pH 5.2. The precipitate was taken up in 0.2 *N* phosphate buffer. This fraction is designated as B2. The remaining solution was then made up to saturation with ammonium sulphate and the resulting precipitate prepared as usual. This preparation is designated Fraction B3.

Total nitrogen was determined using the micro-Kjeldahl method.

All electrophoretic analyses were carried out in a Tiselius apparatus of the type described by Longsworth (2, 3). Temperature was maintained at 2° C. and all analyses were made using a field strength of 4.1 to 4.2 volts per cm.

Results

Extraction of Pea Meal

It was found as a result of preliminary work that it was impossible to get complete extraction of the proteins present in pea meal using salt solutions as dispersing agents. The protein in the unfractionated extract represented approximately 65% of the total nitrogen of the peas. Part of the other 35% was nonprotein in nature and part was not extracted by the 0.2 *N* phosphate buffer. The results reported in this paper obviously refer only to the 65% of the total nitrogen that was in the unfractionated extract.

Fractions A1, A2, and A3, respectively, contained 56%, 24%, and 14% of the protein in the unfractionated extract. Thus it appears that only 6% of the protein was not precipitated during preparation of the fractions. Unfortunately the amount remaining in solution was not checked directly, and since considerable mechanical error is involved in the various stages of

preparation, these figures must be taken as approximations only. According to Osborne and Harris (5), much more protein should have remained in solution after saturation with ammonium sulphate.

Electrophoretic Analyses

In general, duplicate determinations of electrophoretic patterns agreed very well. Since none of the preparations yielded patterns with clearly separated components, it was necessary to interpret the nonsymmetrical nature of the patterns as indicating different components that varied within themselves in electrostatic properties. Mobility is taken as the basic criterion for identifying similar components in the different fractions.

The diagrams for the descending boundaries obtained with the unfractionated extract and with each of the fractions are shown in Fig. 1. There are three distinguishable components in the unfractionated extract with the

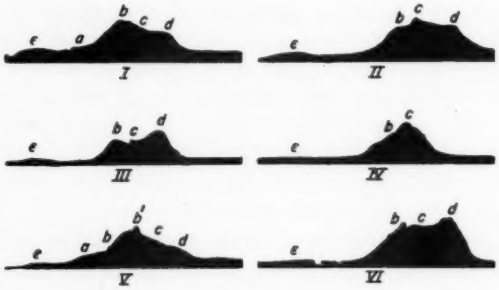

FIG. 1. *Electrophoretic diagrams; pH = 7.8, field strength = 4.1 to 4.2 volts per cm.*
I. Unfractionated extract; protein conc. = 0.86%, time = 15,315 sec.
II. Fraction A1; protein conc. = 1.12%, time = 17,717 sec.
III. Fraction A2; protein conc. = 0.76%, time = 14,570 sec.
IV. Fraction A3; protein conc. = 0.76%, time = 18,418 sec.
V. Fraction B2; protein conc. = 1.04%, time = 16,973 sec.
VI. Fraction B3; protein conc. = 1.56%, time = 19,615 sec.

suggestion that the middle one is actually made up of two. This is clearly shown in the studies of the fractions. The mobility of these components is included in Table I, which summarizes the mobility at pH 7.8, and the amount of each component in each fraction. The type of electrophoretic diagram obtained and the difficulty of satisfactorily separating the components make both the mobility values and proportions of each component subject to considerable error.

The mobility values are all negative, and while some variability is exhibited by the values for the same component in different fractions the identification is fairly definite. In earlier work with other more clearly defined components and in work with gluten protein (1) variability of the same order was experienced.

TABLE I

MOBILITY AND AMOUNT OF EACH PROTEIN COMPONENT IN THE VARIOUS PREPARATIONS

Fraction	Mobility,* 10^5 cm.^{2}/volt/sec.				% of total protein			
	Component				Component			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*
Unfractionated	2.19	4.50	—	6.14	9	61	—	29
Fraction A1	—	4.58	5.12	6.37	—	31	32	37
" A2	—	4.13	5.03	6.45	—	28	15	56
" A3	—	4.09	5.17	—	—	32	67	—
" B2	2.14	4.32**	5.16	6.10	11	52**	20	17
" B3	—	4.24	4.82	6.16	—	37	22	42

* *All mobilities are negative.*

** *Fraction B2 appears to include another component (b^1), with mobility of 3.82, making up 30% of the total protein. This component was reproducible in different runs.*

The slowest component appeared only in the unfractionated extract and in Fraction B2. Actually B2 should contain the same components as A1 plus A2 unless the dilution step in preparation had a pronounced effect on the solubility of one or more of the components. There appears to be a marked effect on Component *a* since it was apparently not precipitated when dilution preceded salting out, but was precipitated when the dilution step was omitted. This component is probably an albumin-type protein, and may well correspond to the legumelin of Osborne (5).

Component *b* makes up a substantial part of each preparation, while *c* is clearly defined in each fraction. These two components are much alike in behavior, but the variability in proportions is difficult to explain. On the average they appear to make up about 60% of the total protein of the extract.

The appearance of the b^1 component in Fraction B2 was at first attributed to faulty technique. It appeared, however, to be reproducible. Thus, the precipitation by 60% saturated ammonium sulphate solution not only affected Component *a*, but apparently caused a separation of some of the other components. It seems not unlikely that the whole system represented by Components b^1, *b*, and *c* is a typical Sørensen protein complex (6), with gradual variation in properties of the components. It also seems probable that the method of preparation affects the separation of these components under the influence of an electric current.

Component *d* is a fast-moving component and is clearly present in all preparations except A3. The difference between A3 and B3 must again be attributed to the effect of the preliminary dilution step in the preparation of the A fractions. It is not at all clear why Component *d* should be absent from A3 and yet be the largest component in B3. In any case, there seems to be no doubt that *d* is a clear-cut component distinguishable from all others, and accounting for approximately 30% of the protein in the pea meal extract.

In only one fraction does there seem to be good separation of components. Fraction A3 is made up wholly of Components *b* and *c*, which, as already suggested, may be considered to be components of a single system. The specificity of solubility is not high, however, since these components are not clearly separated from *d* in the preparation of Fraction B3.

In the preparation of the fractions discussed above, the solution from which precipitation occurred was always adjusted to a pH of 5.2. The pH of the precipitation medium is likely to affect the amounts and proportions of the different components precipitated, so an experiment was conducted to test this effect. Four preparations were used and treatment was identical except for the pH to which the extract was adjusted at the time of precipitation. The four pH levels used were 6.75, 5.50, 5.20, and 4.20. Ammonium sulphate was added to make the solution 60% saturated, and the precipitates prepared are for the other fractions.

Electrophoretic diagrams of the four preparations are given in Fig. 2. All runs were made at pH 7.70 ± 0.06, while protein concentration varied from

FIG. 2. *Electrophoretic diagrams of pea protein precipitated at 60% of saturation with ammonium sulphate; pH at time of analysis = 7.8; field strength = 4.1 to 4.2 volts per cm.; protein concentration approx. = 1%; time approx. = 16,000 sec.; pH at time of precipitation: I = 6.75, II = 5.50, III = 5.20, IV = 4.20.*

0.83 to 1.10%. The mobility values and proportion of each component are given in Table II. The preparation carried out at pH 5.2 should yield results

TABLE II

MOBILITY AND AMOUNT OF EACH PROTEIN COMPONENT AS AFFECTED BY pH OF THE PRECIPITATION MEDIUM

pH at precipitation	Mobility,* 10^5 cm.^{2}/volt/sec.				% of total protein			
	Component				Component			
	a	*b*	*c*	*d*	*a*	*b*	*c*	*d*
6.75	2.33	4.11	5.26	—	14	62	16	6
5.50	2.19	4.15	5.30	6.46	10	51	22	17
5.20	2.01	3.90	5.11	6.02	14	55	17	16
4.20	2.12	4.01	5.14	6.18	11	40	20	30

* *All mobilities are negative.*

in agreement with those for Fraction B2. Within acceptable limits of error this agreement is attained, if Components *b* and b^1 of Fraction B2 are considered to correspond to Component *b* in Fig. 2. The low mobility for B suggests that this is really a mean value for b^1 and *b*. There is no evidence of b^1 in the preparations precipitated at 6.75 or 5.50, but more than a suggestion of this component in the other two diagrams.

Electrophoretic separation of the three Components *b*, *c*, and *d* is progressively improved as the pH of the precipitation medium decreases. Component *a* seems to be entirely insensitive to this treatment; the amount of *c* seems to remain about constant; but the amount of *d* increases progressively while that of *b* decreases as the pH decreases. The determination of the basic cause of these changes will require further study, but there seems to be no doubt as to its importance.

Discussion

The results presented here are preliminary in nature, and have already suggested many experiments that should be performed. They are sufficiently accurate, however, to add to the growing evidence showing the relatively poor separation of plant protein species that can be obtained by variable solubility alone. Since solubility has been used as the main criterion of protein classification, the importance of such evidence cannot be overstressed.

The components of the soluble proteins in pea meal are not clearly defined, but the results of this preliminary work show that distinct types are present, and that probably one of these is albumin in character. Whether the results are interpreted as indicating three or more components depends on the importance attached to the separation effected with the middle components in some preparations. Whatever the interpretation, however, the middle component or components appear to make up a typical Sørensen protein complex varying within itself in properties.

It is very unlikely that Osborne's methods (4, 5) yielded three distinct protein species. There is evidence that there are three different types of protein present in pea meal, but these are certainly not homogeneous, and the properties overlap sufficiently so that separation of types is not accomplished using precipitation methods. Electrophoretic separation of components in protein is much more precise than is any method of separation based on solubility (7). It seems certain, therefore, that Osborne's "three proteins" were mixtures of components, with the proportions of each component varying in the different fractions. Much more accurate work is necessary before the components can be adequately described.

References

1. COLVIN, J. R. and McCALLA, A. G. Physical and chemical properties of gluten. 1. Estimation of molecular properties using electrophoretic and diffusion data. Can. J. Research, C, 27 : 103-124. 1949.

2. LONGSWORTH, L. G. Recent advances in the study of proteins by electrophoresis. Chem. Revs. 30 : 323-340. 1942.

3. LONGSWORTH, L. G. Optical methods in electrophoresis. Ind. Eng. Chem. Anal. Ed. 18 : 219-229. 1946.

4. OSBORNE, T. B. and CAMPBELL, G. F. The proteins of the pea, lentil, horse bean, and vetch. J. Am. Chem. Soc. 20 : 410-419. 1898.

5. OSBORNE, T. B. and HARRIS, I. F. The proteins of the pea (*Pisum sativum*). J. Biol. Chem. 3 : 213-217. 1907.

6. SØRENSEN, S. P. L. The constitution of soluble proteins. Compt. rend. trav. lab. Carlsberg Ser. Chim. 18 (5) : 1-124. 1930.

7. TISELIUS, A. Electrophoresis of serum globulin. II. Electrophoretic analysis of normal and immune sera. Biochem. J. 31 : 1464-1477. 1937.

PHYSICAL AND CHEMICAL PROPERTIES OF GLUTEN
I. ESTIMATION OF MOLECULAR PROPERTIES USING ELECTROPHORETIC AND DIFFUSION DATA[1]

By J. R. Colvin[2] and A. G. McCalla[3]

Abstract

The molecular characteristics of gluten in sodium salicylate solutions have been studied by means of diffusion and electrophoretic techniques. The results of the observations indicate that gluten in sodium salicylate is electrostatically homogeneous with a high mean negative valence of 34 and therefore a high surface charge density. The isoelectric point of gluten in this dispersing agent is below pH 4.0. This low value of the isoelectric point and the high valence of the protein molecule is probably due to selective adsorption of salicylate ions on gluten. The particles in the fraction of gluten molecularly dispersed in 0.5μ sodium salicylate are prolate ellipsoids of revolution of the order of 25Å in diameter and 400 to 450Å long. These are mean values as the molecularly dispersed particles are not uniform in mass. When they are aggregated it is side by side association rather than end to end. The density of dehydrated gluten is 1.291 gm. per cc. Qualitative evidence is given for the view that the degree of hydration of gluten particles is probably not great.

Introduction

The results of an extensive study on gluten in sodium salicylate solutions using ultracentrifugal and diffusion methods were published in 1942 by McCalla and Gralén (26). These results showed that in sodium salicylate solutions gluten existed in two states, molecularly dispersed and aggregated. There was, however, progressive continuous variation in the physical properties and dimensions of both the molecules and the aggregates. This proved conclusively that "gliadin" and "glutenin" did not exist as distinct entities and also gave strong support to the view that gluten is a reversible protein complex, as suggested by earlier work (27,44).

The study mentioned above proved the heterogeneity of gluten in sodium salicylate solutions as far as mass characteristics are concerned but no corresponding study of the electrical characteristics of gluten in sodium salicylate has been made.

Moreover, only a very limited amount of work has been done on gliadin, gluten, or related proteins in other solvents. Wood and Hardy (49) had shown that the physical properties of a gluten ball were a function of the electrical charge on the gluten micelles, which in turn depended on the hydrogen ion concentration of the surrounding medium. They proved that the gluten micelles had a positive charge in acid medium and a negative charge

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in alkali, as was to be expected. Moreover, they could find no difference in the behavior of gluten and of a sample of gliadin. On the basis of these results, they concluded that the physical state of gluten was largely determined by the potential difference between the protein and the dispersing fluid. Kemp (14) and Kemp and Rideal (15) have used the microelectrophoretic technique with gliadin adsorbed on quartz to estimate the isoelectric point of gliadin and to compare the surface charge densities of various flours. They found an isoelectric point of approximately 5.00 for gliadin but this value was inversely dependent on ionic strength. Despite extensive data on the adsorption of gliadin on quartz, few data were given on the surface electrical properties of gliadin itself.

The only study of gliadin using the Tiselius electrophoretic technique is that of Schwert, Putnam, and Briggs (39). They found that gliadin dispersed in acetate buffers was markedly heterogeneous. Two main fractions of widely different mobilities and isoelectric points were identified but these two fractions themselves could be subdivided into components. Because of this heterogeneity and also because of a strong asymmetry between patterns obtained in the two legs of the electrophoretic cell, these workers concluded that gliadin was a mixture of components with widely different surface charges interacting in a non-ionic manner.

While the present study was in progress, Scallet (37) published a report of a study on zein in aqueous alcohol using the Tiselius electrophoretic apparatus. As the result of multiple fractionations followed by electrophoretic analysis he concluded that zein was a typical association–dissociation system as described by Sørensen (42).

Since electrophoretic data naturally supplement ultracentrifugal and diffusion data, the present study was undertaken. It seemed logical to suppose that the known variation in the chemical composition of gluten fractions (27) would be reflected in their electrical behavior and information on such variability would lead to a better understanding of the mechanics of the gluten complex. Specifically, it was hoped to determine the number of components in gluten and the electrophoretic mobilities of the components. Later, this objective was broadened to include the charge density of the molecularly dispersed gluten particle. These data, with existing ultracentrifugal and diffusion values, might then allow an estimate of the size and degree of hydration of the molecule.

Materials and Methods

The gluten used throughout this study was obtained from an unbleached high quality flour milled from hard red spring wheat. The ash content of this flour was 0.45% and the protein content, 14.2%. When baked by a standard malt–phosphate–bromate formula this flour gave high quality loaves of good volume and texture. The gluten itself was in every way typical of that from the best Canadian spring wheat, being firm, elastic, and extensible.

All sodium salicylate used was of C.P. reagent grade. Unless otherwise stated, all solutions were of 0.5 ionic strength (0.5μ), which corresponds to slightly less than 8% sodium salicylate by weight. All preparations were checked before use against a standard solution by a conductivity apparatus.

Because McCalla and Gralén had found that gluten dispersed in sodium salicylate contained both molecularly dispersed protein and aggregates, the electrophoretic properties of two gluten preparations were investigated, i.e. molecularly dispersed gluten and whole gluten. Each preparation was represented by four samples independently prepared from the flour in as uniform a manner as possible and stored under constant conditions. The molecularly dispersed series was prepared as described by McCalla and Gralén for their CIV fraction except that the final disperson was made in 0.5μ solution rather than in 12% sodium salicylate. This fraction is that portion of the gluten protein that remains in solution in 8% sodium salicylate made up to 8% of saturation with magnesium sulphate, but is precipitated when the concentration of magnesium sulphate is raised to 20% of saturation. Each of the samples in the "whole gluten" series was prepared by washing the starch from 100 gm. of ether-extracted flour with Dill and Alsberg's buffer (7) for 10 min. The resulting gluten ball was immediately torn into small pieces and allowed to disperse in a liter of sodium salicylate solution for three days with occasional gentle shaking. The undispersed gluten was then removed by centrifuging and the opaque solution clarified by adding magnesium sulphate until the solution was 1.5% saturated with respect to this salt. After removal of the precipitate by a second centrifugation, the solution was diluted to a protein content of from 0.6% to 0.8% and stored at 5° C. until used.

Electrophoretic mobilities and patterns were obtained at 2.0° $\pm$ 0.05 using a Tiselius apparatus described by Longsworth (21). Details of dialysis of sample, cell manipulation, and instrument handling were essentially as given in papers by Longsworth (19, 21), Longsworth and MacInnes (24, 25), and Svensson (46) except for the following modifications. It was found that the power supply using a No. 80 rectifier tube could not maintain a constant voltage at the relatively high currents needed (20 to 25 ma.). Accordingly, B batteries in series were substituted as a source of potential. This arrangement held the voltage satisfactorily constant. Longsworth (20) has described the use of a cell that eliminates errors in mobility due to movement of the rubber sleeves on the original Tiselius apparatus. Since no Longsworth cell was available, it was necessary to use a Tiselius cell. This necessity has been reflected in the lack of precision in the pH-mobility curves (see Fig. 3) and is the greatest single source of error in this study. It was accentuated by the low mobility of the gluten in the buffer.

Mobilities were calculated using the method of Longsworth and MacInnes (25) for locating the boundary and the formula $\mu = \frac{Vk}{it}$. Since no micro-comparator was available, the negatives were enlarged approximately four times and measurements were made directly on these enlargements. By this

method, boundaries could be located within 0.01 cm., which with a boundary travel of 1.5 cm., represents an error of 0.6%. This is well within the limits of error set by the source mentioned above.

Conductivities were measured using an Industrial instrument, which on testing with a calibrated cell showed an accuracy of ± 1%. This comparatively low order of accuracy had to be used since no better instrument was immediately available. The relative error due to this factor was probably reduced by the fact that the conductivity of the 0.5μ solution was quite constant at the given temperature for different samples.

Hydrogen ion concentrations were measured with a Beckmann Model G pH meter to ± 0.05 pH units.

It will be noticed that no buffer salts were used in addition to the main electrolyte. This was because repeated preliminary tests showed that no changes in hydrogen ion concentration took place in the boundary area even after 10 hr. of passing a 25 ma. current.

During the course of the study it was found necessary to determine the diffusion constant of molecularly dispersed gluten at 2.0° ± 0.05 in sodium salicylate. This was done by supporting a Neurath cell (30) in the thermostat of the electrophoresis apparatus at a position where the magnification was unity and using the formula $D = \frac{A^2}{4\pi t (H_m)^2}$ for calculation from the patterns obtained, as used by Rothen (36). With a polydisperse substance such as gluten, this formula yields only a mean value for the diffusion constant but this was satisfactory for a first approximation. A test of the method on 0.1 *M* sucrose at 20° gave a mean diffusion constant from 12 determinations of $(44.3 \pm 0.9) \times 10^{-7}$ moles/cm.^{2}/sec. as compared with the value from International Critical Tables of $(44 \pm 2) \times 10^{-7}$ moles/cm.^{2}/sec.

Viscosities were determined using an Ostwald viscosimeter calibrated against water at 2.0° ± 0.05. Techniques were as described by Findlay (8).

After this investigation was nearly complete, it became apparent that it would be useful to know the density of dry gluten. No estimate of this constant could be found so an attempt was made to determine it by the methods of Neurath and Bull (31) for egg albumin. Their materials and technique were followed closely throughout except in the preparation of the sample.

As a means of estimating the relative importance of starch and electrolyte contamination in the final product, the gluten for the density determination was prepared by two methods each represented by five independent samples.

In Method 1, the gluten ball as obtained from 20 gm. of ether-extracted flour was merely washed extremely thoroughly with distilled water until only the faintest traces of starch were apparent in a sedimentation test. In general, this required more than an hour per sample. The thoroughly washed gluten was then frozen and, while frozen, ground to a coarse powder. The water was removed from this frozen gluten by lyophilization in a high vacuum over

P_2O_5. Throughout this process, the sample was kept frozen by solid CO_2. The resulting coarse granules, which retained the natural color of gluten, were powdered in a mortar and stored over P_2O_5 until used. The original properties of the gluten could be recovered by the addition of water to this powder.

In Method 2, the gluten was washed from 20 gm. of ether-extracted flour in the ordinary manner and dispersed for three days in sodium salicylate. The undispersed material was then centrifuged off and the opaque solution, now considered to be free from starch, made up to 20% saturation with magnesium sulphate. The gluten precipitated by the magnesium sulphate was collected on a stirring rod and washed as thoroughly as possible with distilled water to remove the electrolytes. It was then frozen and lyophilized in the same manner as for the undispersed series.

Throughout this study, all protein concentrations were estimated by a micro-Kjeldahl apparatus accurate to $\pm$ 1% of the total nitrogen present.

Experimental Results

Preliminary Experiments on Egg White Proteins

Before undertaking work on gluten, it was thought advisable to check apparatus and technique by duplicating previous work on a well-known protein. For this purpose, the work of Tiselius on egg albumin (47) was taken as reference except that the egg albumin was prepared according to the method of Kekwick and Cannan (13). The isoelectric point of the present preparation of egg albumin was found to be shifted 0.15 pH units to the acid side as compared with that of Tiselius (Fig. 1), which is three times greater than the

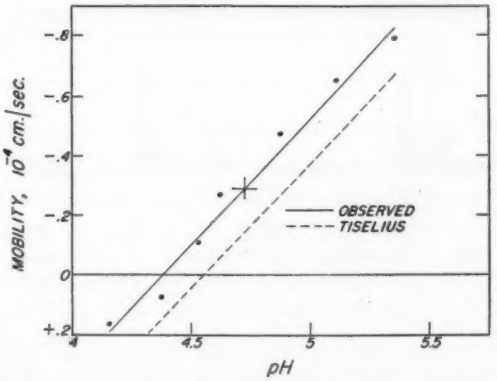

FIG. 1. *A comparison of the observed pH mobility curve for egg albumin in 0.02 M acetate buffer at 20° C., with that of Tiselius for the same protein under the same conditions.*

error involved in reading the pH meter. Moreover, tests showed this discrepancy to be reproducible. In spite of this, the results were taken as indicating the general reliability of our methods because the slopes of both

curves were identical. This could only be so if there were no serious errors in methods of observation and calculation. It was assumed that the difference in isoelectric points was due to small differences in the original material or to differences in preparation. It is unlikely that the difference is due to errors in the standard buffer solution because this was made up according to Clark (6) and checked against commercial tablets.

Since sodium salicylate is a denaturing agent for some proteins (4) and must be used in rather high concentrations for gluten, the possibility of its altering or obscuring some of the properties of the protein must be borne in mind. Rose and Cook (35) have shown that this reagent has only a slight effect on the properties of gluten as compared with the effects of the more usual dispersing agents, while Spencer and McCalla have demonstrated that there is no permanent alteration in the character of gluten after dispersion in salicylate (44). This does not prove that there is not a temporary modification of the surface properties, particularly surface charge densities, while the gluten is dispersed. To demonstrate the effect of sodium salicylate solutions on a well characterized protein (23) the patterns of egg white in this reagent and in Sørensen's phosphate buffer, which is widely used as a buffering agent, were obtained. The patterns for this mixture of proteins are given at 0.1 *M* and at 0.5 *M* for both solutions in Fig. 2. An inspection of these patterns shows

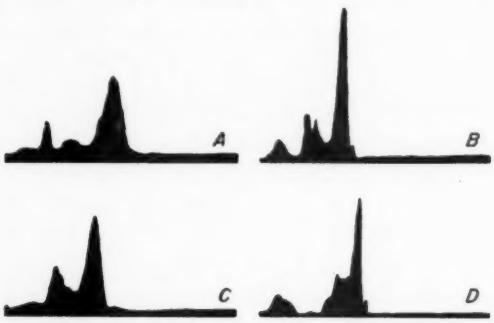

FIG. 2. *Electrophoresis diagrams of egg white. Protein concentration = 1.00% in each test.*

A. In 0.1 M phosphate buffer at pH 6.9. Time = 60 min.
B. In 0.1 M sodium salicylate at pH 6.9. Time = 60 min.
C. In 0.5 M phosphate buffer at pH 7.0. Time = 200 min.
D. In 0.5 M sodium salicylate at pH 7.0. Time = 330 min.

that the number of components of egg white in sodium salicylate is the same as that in phosphate buffer. Moreover, the separation of components in sodium salicylate is equally as sharp as that in phosphate. A comparison of the patterns at the two concentrations of both reagents indicates that the only effect of the increased ionic strength is a reduction in mobility, as might be expected. From this it was concluded that, for the proteins of egg white, sodium salicylate is at least as satisfactory a buffer as Sørensen's phosphate.

Unfortunately, a similar direct comparison of the two solutions for gluten is not possible owing to the insolubility of gluten in most neutral solutions. The assumption has to be made that sodium salicylate has no more specific effects on gluten than on egg white proteins.

Main Electrophoresis Experiments

Following the completion of the preliminary experiments and a number of trial runs, pH-mobility curves for both the whole gluten and the molecularly dispersed gluten were determined. It was, of course, possible to obtain electrophoretic patterns for each series at the same time. The results of this study

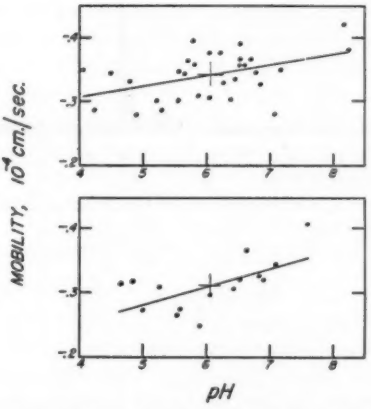

FIG. 3. *Mobility of gluten dispersed in 0.5 M sodium salicylate as a function of pH.*
Top—whole gluten.
Bottom—molecularly dispersed gluten.

are plotted in Fig. 3. Representative electrophoresis patterns for whole gluten and molecularly dispersed gluten are given in Fig. 4.

A study of Fig. 4 shows that both the whole gluten and the molecularly dispersed portion are electrically homogeneous in sodium salicylate. The patterns are asymmetrical to a small degree in some cases but no patterns were obtained in which a separation of the peak could not be correlated with an error in technique. The only conclusion possible is that in sodium salicylate all fractions of gluten are essentially the same electrostatically. This does not apply to all portions of the nitrogenous constituents of wheat, however. It will be noticed that the patterns exhibit a small peak migrating faster than the main boundary. This was a constant feature of all patterns and its presence is proof that if major components are present in gluten, this technique should discover them. That the small peak or boundary is not a major fraction of gluten is proved by its size alone; it represents only a very small fraction of

the total protein present and therefore cannot be important in the complete complex. It has been identified tentatively as an impurity in the sample due to the presence of nongluten protein (26).

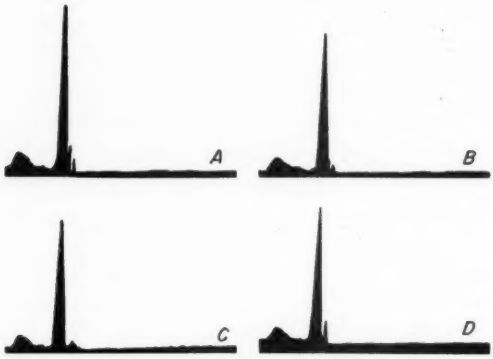

FIG. 4. *Electrophoresis diagrams of gluten in 0.5 M sodium salicylate. Field strength = 2.3 volts per cm. Time = 250 min.*
A. Whole gluten, protein conc. = 0.65%, pH = 6.3.
B. Whole gluten, protein conc. = 0.65%, pH = 7.2.
C. and D. Molecularly dispersed gluten, protein conc. = 0.5%, pH = 5.0.

The results in Fig. 3 show that within the pH range studied, 4.00 to 8.25, gluten carries a negative charge in sodium salicylate solution. Moreover, the size of this charge is remarkably insensitive to changes in pH. This is shown by the very low slope of the regression line. This low slope is probably due to the fact that it was not possible to work in the isoelectric range because salicylic acid precipitates from the solution at pH values less than 4.0. All pH-mobility curves approach zero slope at hydrogen ion concentrations remote from the isoelectric point. Independent evidence for the existence of an isoelectric point for gluten in sodium salicylate at a pH less than 4.0 was given by the strong tendency for the gluten to precipitate at pH values less than 5.0. Below pH 5.0 it was difficult to keep the gluten in solution although this tendency to settle out was not accompanied by the precipitation of salicylic acid until pH 4.0 was reached. This very low isoelectric point of gluten in sodium salicylate will be referred to again in connection with the high value of the surface charge density.

A comparison of the two sections of Fig. 3 shows that the slopes of the regression lines are approximately the same for whole gluten and for the molecularly dispersed fraction. This is confirmed by statistical analysis* and this fact corroborates the suggestion from the electrophoresis patterns that the surface charge characteristics are the same in both cases, since they react similarly to changes in the medium. Statistical analysis also proves**

*$t = 0.98$; D.f. = 41.
**$t = 2.36$; D.f. = 43.

that the mean mobility at any given pH is lower for the molecularly dispersed fraction than for whole gluten. Since the mean particle size is lower for the molecularly dispersed fraction (26) and the evidence given above indicates that the surface charge densities are approximately equal, this lower mobility must be a reflection of a larger frictional ratio $\frac{f}{f_0}$. This is in agreement with the observation of McCalla and Gralén that the most soluble fraction of gluten has the largest frictional coefficient. Assuming constant hydration, this in turn must mean that the particles making up the most soluble portion of gluten have the greatest length to breadth ratio.

These two experiments support two conclusions: 1. That in sodium salicylate solutions, gluten is an electrostatically homogeneous substance. 2. That the isoelectric point of gluten in sodium salicylate is somewhere below pH 4.0. Schwert, Putnam, and Briggs (39) obtained results on gliadin so much at variance with our conclusions concerning the nature of gluten that it was thought worthwhile to repeat their study using gluten from our flour. It proved possible to duplicate their work in all respects but one. Even at field strengths in excess of those mentioned in their report no multiple separation of the two main components into lesser complexes could be obtained. All other observations made by these authors were confirmed, however, proving that the source of the discrepancy does not lie in the material used. According to this evidence, the fundamental mechanisms of dispersion in acetic acid and in sodium salicylate must be different.

While this study was in progress, Scallet (37) published a report on the electrophoretic analysis of zein, in which he observed that as the ionic strength increased, separation of components in the mixture decreased. It has been suggested that this suppression is the cause of the apparent electrophoretic homogeneity of gluten in sodium salicylate. To test this point as far as physical conditions would permit, a dispersion of gluten in 0.5μ sodium salicylate was diluted until the protein was on the verge of precipitation. The final concentration of sodium salicylate was 0.3μ. An electrophoretic analysis was then made on the sample at this concentration. The results are shown in Fig. 5.

FIG. 5. *Electrophoretic diagrams of whole gluten in 0.3 M sodium salicylate. Field strength = 3.8 volts per cm., time = 250 min., protein conc. = 0.35%, pH = 6.0.*

It will be observed that this plate gives no more evidence for the heterogeneity of gluten than do those conducted at 0.5μ. One main peak only is present. Since 0.3μ is still a fairly concentrated solution and because the altered ionic strength may have changed the degree of molecular dispersion (26), this experiment is not conclusive but taken in conjunction with the presence of the nongluten protein boundaries, it is a strong indication that the homogeneity of gluten is not due to suppression by high salt concentration.

It was also thought that the failure to resolve components in gluten might be due simply to the low mobility in the high salt concentrations. If this were so, increasing the current–time product should tend to separate components. Accordingly, an experiment was conducted on whole gluten in 0.5μ sodium salicylate using as high a current as the thermostat would allow for as long a period as the electrode capacity would permit. The results are shown in Fig. 6.

FIG. 6. *Duplicate electrophoretic diagrams of whole gluten in 0.5 M sodium salicylate. Field strength = 1.7 volts per cm., time = 600 min., protein conc. = 0.5%, pH = 6.7.*

These patterns are in every way comparable to those obtained under the less rigorous conditions and give no support to the above suggestion.

Summing up all the evidence, the most reasonable interpretation is that sodium salicylate does not seriously affect the separation of components. The work with egg white proteins, nongluten protein constituents, and with low concentrations of electrolytes supports this view. It must be pointed out, none the less, that this evidence is suggestive only, not conclusive. Sookne and Harris (43), working with the proteins of silk have shown that the more complex ions such as phthalate and picrate are preferentially adsorbed, sometimes to the point where the isoelectric point disappears from the range studied. If gluten constituents should selectively adsorb salicylate ions, it might raise their electronegativity to a point where smaller initial differences are not distinguishable. In fact, evidence of just such a mechanism is presented in the next section of this paper. It seems that a salicylate–gluten complex is formed, which is one step in the dispersion of the gluten mass. If this is so, it must mean that the surface characteristics of the gluten molecule or micelle are very different from those of egg white proteins and this in turn must be related to their respective amino acid compositions. That wide differences in the amino acid composition of the two proteins exist is well known (5) but as yet we know nothing definite as to how these differences may be reflected in the nature of the surface of the molecule. This question deserves much greater study.

Diffusion

The values obtained from 13 determinations of the diffusion constant of molecularly dispersed gluten at 2.0° ± 0.05 are given in Table I. In the last column of this table, the diffusion constants are corrected to 20.0° C. in pure water in order to provide a comparison with previously published values (26) but for all calculations made in this paper, the diffusion constant is that for 2.0° in 0.5μ sodium salicylate. It will be observed that, in general, the

TABLE I

DIFFUSION CONSTANT OF MOLECULARLY DISPERSED GLUTEN AT 2.0° C.

Run No.	Time (min.)	D × 10^7	D, corrected to 20° in pure water, × 10^7
1	510	2.13	5.23
	1056	2.06	5.06
2	564	1.98	4.86
	762	1.89	4.64
	1333	1.97	4.83
3	518	2.13	5.23
	787	1.96	4.81
4	267	2.00	4.91
	503	1.97	4.83
5	248	2.28	5.60
	698	1.97	4.83
	1371	1.91	4.69
6	328	1.98	4.86

longer periods are associated with the lower diffusion constants. This is because some slight mixing of solutions is unavoidable when the boundaries are formed. The effect of mixing is to raise the apparent diffusion constant but it is obvious that the effect will approach zero as the time of diffusion approaches infinity. In order to approximate this condition the regression line of a plot of $\frac{1}{t}$ against the diffusion constant was calculated and the "true" diffusion constant taken as the value of the intercept of the regression line on the diffusion axis. This value is thus an estimate of the diffusion constant of the material after a very long time has elapsed since the formation of the boundary. Corrected in this manner, the mean diffusion constant of molecularly dispersed gluten in 0.5μ sodium salicylate at 2.0° was estimated as $(1.91 \pm 0.09) \times 10^{-7}$ moles/cm.^{2}/sec.

It cannot be emphasized too strongly that the value of the diffusion constant obtained here is a mean value for the molecularly dispersed portion of gluten and therefore any particular part of this fraction may be expected to yield values that differ from the figure given. Since gluten is a polydisperse system (26), the same limitation applies to practically all numbers that are used to describe any appreciable part of the complex. These numbers are intended to give a reliable description of the protein, not to furnish exact magnitudes for a particular case.

Density of Gluten

The results of a determination of the density of dry gluten prepared by the two methods described previously are given in Table II. It is evident from

TABLE II

DENSITY OF DRY GLUTEN AT 20° C., GM./CC.

Series	Sample	Density	Mean
Washed	1	1.299*	1.310
	2	1.312	
	3	1.311	
	4	1.310	
	5	1.309	
Reprecipitated	1	1.296	1.291
	2	1.292	
	3	1.288	
	4	1.291	
	5	1.288	

*Rejected on a statistical basis according to Willard and Furman (48, p. 67).

inspection that the two series must differ and this opinion is sustained by statistical analysis.* Since the washed samples were known to contain very small amounts of starch, which has a density of 1.50 (11), the mean of the values for the reprecipitated series was taken as the better estimate of the density of dry gluten.

Viscosity of Dispersions

The values for viscosity of the dispersions of gluten in sodium salicylate as used in the other studies, and two values for the viscosity of 0.5μ sodium salicylate as determined from independent solutions are given in Table III.

TABLE III

VISCOSITIES OF DISPERSIONS, 2.0° C.

Series	Sample	Viscosity (centipoises)	Protein conc., %
0.5μ Sodium salicylate	1	2.056	—
	2	2.054	—
Whole gluten	3	2.503	0.86
	4	2.482	0.87
	5	2.546	0.98
	6	2.461	0.84
Molecularly dispersed gluten	7	2.310	0.61
	8	2.312	0.62
	9	2.319	0.62
	10	2.320	0.61

Each value listed is the mean of at least five trials. Also listed are protein concentrations of the dispersions as calculated from mgm. of nitrogen per cc. These represent the limiting factor in the accuracy of these determinations.

*t = 10.9; D.f. = 7.

There is, as pointed out by Abramson, Moyer, and Gorin (1, p. 137), a difficulty in the physical interpretation of viscosity as determined in the laboratory. In very dilute solutions of spherical proteins at their isoelectric point, the viscosity term occurring in the electrophoretic and diffusion equations "is clearly that of the medium and is equivalent to the coefficient of viscosity as determined in the laboratory". When the solutions become more concentrated and particularly when the particles are both highly charged and highly anisometric, this is no longer true. Instead of the protein molecule moving through a medium of pure dispersing agent, it now moves through a protein-solvent solution and the forces to which it is subjected may be appreciably different. This question has been discussed by Lauffer (17). He concludes from a study of the dependence of sedimentation constants, diffusion constants, and electrophoretic mobilities on concentration of the solution, that "there is considerable evidence that in the diffusion process and in electrophoretic migration a solute molecule must be considered to move through a medium with the viscosity of the solution rather than through one with the viscosity of the solvent". On the basis of this evidence then, the viscosity of the whole solution rather than that of the medium has been adopted as the closer approximation to the truth.

Data for Electrical Charge on Gluten

After several unsuccessful attempts to determine the charge on the gluten molecule by means of membrane potentials (2, 3) the moving boundary method as suggested by Longsworth (22) and Svensson (46) was modified for use with gluten in sodium salicylate. Since Longsworth had developed the theory in greater detail and checked it against other methods for egg albumin, his procedure was followed. Essentially, for a uni-univalent electrolyte, the application of this method depends on the determination of the relative mobilities of the protein, the buffer cation, and the buffer anion, together with the change in refractive index per equivalent of buffer and the change in refractive index at the buffer-concentration boundary (Longsworth's nomenclature) per unit concentration of protein in the moving boundary.

Then, by application of the formula taken from Longsworth (22)

$$\eta^{\beta} - \eta^{\gamma} = K_{AR}\left(\frac{r_B - r_R}{r_B} \cdot \frac{r_A}{r_A - r_R} - \frac{1}{2}\right) 10\rho e$$

where

$\eta^{\beta}-\eta^{\gamma}$ = change in refractive index at buffer-concentration boundary
K_{AR} = change in refractive index per equivalent of electrolyte
r_B = relative mobility of protein
r_R = relative mobility of anion
r_A = relative mobility of cation
ρ = grams protein per 100 ml.
e = charge in Faraday equivalents per gram of protein, after substitution of the appropriate constants, it is possible to calculate e. If the molecular weight of the protein is known, this allows an estimate of the

"valence" of the protein ion at the selected pH. Since most of these constants for sodium salicylate are not in the literature, it was necessary to determine them.

The K_{AR} value for sodium salicylate at 2.0° was estimated by an Abbé refractometer used on 10 samples of solution of progressively varying concentration. The value found for this constant was 0.03417 ± 0.00012.

The absolute mobility of the protein ion can be found from the electrophoretic measurements but in order to find its relative mobility it was necessary to know the mobilities of the sodium and salicylate ions at 2.0° in 0.5μ sodium salicylate. Such values are not given in the literature and the apparatus necessary for ascertaining them directly was not available. Accordingly, it was necessary to calculate values from data for these ions at infinite dilution and 25° (9, 10, 18) on the following assumptions:

1. That sodium salicylate is a strong electrolyte and completely dissociated.
2. That the transference numbers of the Na^+ and salicylate ion are practically constant with variation in temperature.
3. That the transference numbers of Na^+ and salicylate ion are affected only slightly by change in concentration.

Assumption 1 is probably true but it is well known that Assumptions 2 and 3 are not valid in general (28). Independent evidence for the validity of 3 in the case of sodium salicylate was obtained from the stationary character of the buffer concentration boundaries in routine electrophoresis experiments. These boundaries remain practically motionless over a period of four hours and this can only occur when the transference numbers of the ions are the same in both solutions (46). The correctness of Assumption 2 is more questionable but Getman and Daniels (9) and Glasstone (10) show that the variation is small, except for the most highly hydrated ions, over a wide temperature range. Therefore, for a first approximation and as a working hypothesis, it is believed that these two simplifications are justified. Using these assumptions and the published values for the absolute mobility of the sodium ion at 25° and infinite dilution (9) the relative mobilities of the three ions involved were calculated.

The determination of the change in refractive index at the buffer-concentration boundary per unit concentration of protein proved more difficult. The lack of a hollow prism made it impossible to apply Longsworth's procedure (21, 22). Moreover, because of the extremely small quantities involved, efforts to measure them directly failed. It was necessary to resort to an indirect method as follows: under the same conditions of temperature, magnification, and type of cell as in electrophoresis, large schlieren patterns due to a boundary between water and a 0.1 *M* solution of sucrose in water were obtained. The method was essentially that used by Rothen (36) in his adaptation of the electrophoretic apparatus for studying diffusion of ribonuclease. Since the areas of these patterns as measured by a planimeter are

proportional to the differences in the refractive index of the two solutions at the boundary, which can be measured by a refractometer, it is possible to associate a unit area under a curve with a fixed difference in refractive index at the boundary (all other conditions remaining constant). Therefore, the area under a buffer-concentration boundary peak as obtained in routine measurements, multiplied by the constant obtained as above, gives the value of the refractive index change at the boundary. This change in refractive index can then be correlated with the protein concentration of the solution in the cell as determined by a Kjeldahl procedure. Since the relationship is linear for dilute solutions of proteins (22), the slope of this line gives the refractive index change per unit concentration of protein at the buffer-concentration boundary, which is the parameter required.

The experimental values obtained by the foregoing method are plotted in Fig. 7. The calculated regression equation is $\Delta n = 10 \times 10^{-6} + 331.5 \times 10^{-6}$ mgm. N/cc. with a standard error of estimate of 17×10^{-6}.

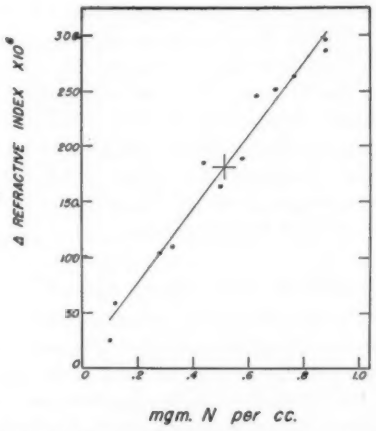

FIG. 7. *Change in refractive index at the buffer concentration boundary as a function of nitrogen content of the solution.*

Thus, within the limits of experimental error, the line passes through the origin as it should. The slope of this line is $(331.5 \pm 18.5) \times 10^{-6}$ per mgm. N/cc., which, assuming a nitrogen factor for endosperm proteins of wheat of 5.70 (12), is equal to $(581.6 \pm 32.5) \times 10^{-6}$ per gram of protein per 100 ml.

Calculated Results

Molecular Charge

The foregoing experimental data permit the calculation of the mean charge of the molecularly dispersed gluten particles as outlined previously. The

constants used and the results of the application of Longsworth's formula are summarized below:

DATA FOR CALCULATION OF MOLECULAR CHARGE

$\Lambda_{(0.5\mu)}$ of sodium salicylate at 25° = 49.8
Λ_0 of salicylate ion at 25° = 37.1
Λ_0 of sodium ion at 25° = 50.1

Absolute mobility of sodium ion at infinite dilution and 25° = 5.2×10^{-4} cm./sec./volt.
Ratio of conductivity of 0.5μ sodium salicylate at 2° and 25° = 0.557.

$\eta^{\beta}-\eta^{\gamma} = 331.5 \times 10^{-6}$ $\rho = 0.570$ gm./100 ml.
$K_{AR} = 341.7 \times 10^{-4}$ $r_A = +1.000$
$r_R = -0.741$ $r_B = -0.188$

Mean charge per molecule = 34 on the assumption that mean molecular weight is 44,000 (26).

This calculation gives the mean "valence" of molecularly dispersed gluten at pH 6.00 in 0.5μ sodium salicylate as 34. From the low slope of the curve shown in Fig. 3 it is evident that this is an estimate of molecular charge over a considerable range of pH. For a protein of molecular weight 44,000, this valence is high. Egg albumin with a comparable molecular weight varies from 0 to −14 over a similar interval. This is a further indication of possible specific ion effects of salicylate on gluten (43). The reversible adsorption of negative salicylate ions on the gluten micelle may raise the electronegativity of the molecule to the observed high value. This would also explain the displacement of the isoelectric point so far towards the acid range and the observed flattening of the pH-mobility curve.

Estimation of Molecular Weight from Diffusion and Viscosity Data

It is possible to check the internal consistency of the data listed by a calculation of the molecular weight of the protein. Polson (33) and Svedberg and Pedersen (45) have shown that if the diffusion constant, the partial specific volume, and the frictional coefficient of a particle are known, a reasonably accurate estimate of the molecular weight is possible from the formula

$$\frac{f}{f_0} = \frac{RT}{D} \cdot \frac{(4\pi N)^{\frac{1}{3}}}{6\pi\eta N (3\,\mathrm{m}^{V})^{\frac{1}{3}}}$$

$\frac{f}{f_0}$ = frictional coefficient
R = gas constant = 8.313×10^7
T = temperature, ° K
D = diffusion constant
η = viscosity of solution
N = Avogadros number
M = molecular weight
V = partial specific volume.

Using the mean value of $\frac{f}{f_0}$ for the molecularly dispersed portion of gluten from McCalla and Gralén (26) and their value for V in 8% sodium salicylate (corrected for the difference in temperature according to Svedberg and Pedersen (45)) with the constants listed in the foregoing tables, a molecular weight of 48,000 was found for molecularly dispersed gluten. This is in satisfactory agreement with the ultracentrifugal value of 44,000 (26) and indicates the general reliability of the constants used.

These data also furnish reliable evidence against the hypothesis that gluten is a highly hydrated sphere. It is possible to explain the extremely large observed $\frac{f}{f_0}$ value on the basis of a sphere with a thick shell of water. Using the value of the diffusion constant given and the formula

$$D = \frac{kT}{6 \pi \eta r}$$

where D = diffusion constant
k = Boltzmann's constant
T = temperature, ° K
η = viscosity of solution
r = radius of particle

r is found to be 45.6Å. From the density data given, an unhydrated gluten molecule of molecular weight 44,000 corresponds to a sphere of radius 23.8Å. Therefore in order to explain the observed diffusion constant by hydration of a sphere, it is necessary to postulate the adsorption of a shell of water approximately 20Å in thickness. Such a shell is extremely improbable on the basis of electrostatic forces alone (1, p. 151) and therefore the data must be interpreted on the assumption of rather large asymmetry of the molecule. Such asymmetry may be represented by two models: (*a*) the oblate ellipsoid, (*b*) the prolate ellipsoid. Simha has shown that for proteins in general and for gliadin in particular, the prolate ellipsoid is the much more probable model (41). On the basis of this evidence, therefore, it is concluded that an elongated ellipsoid of revolution is a reasonable approximation to the shape of the gluten molecule.

Molecular Length–Breadth Ratio

The frictional coefficient $\frac{f}{f_0}$ has sometimes been used as a parameter for the estimation of the axial ratios of molecules. This procedure is not valid in general since the coefficient is a function of the degree of hydration of the molecule as well as its asymmetry. The two effects cannot be separated by a single measurement except for the special cases of zero hydration or completely spherical molecules (45). Polson (33), and more recently, Simha (40) have given equations from which an estimate of the axial ratios of anisometric molecules can be obtained from viscosity data. Polson's equation is an

empirical generalization of Kuhn's equation (16) for rod shaped particles and he has shown that it gives results in good agreement with experimental values. Simha's equation is an extension of Einstein's relation

$$\frac{u - u_0}{u_0} = v\,c\,.$$

where u = viscosity of solution $\quad u_0$ = viscosity of solvent

c = volume fraction of solute $\quad v$ = is a shape factor

to the case where the particles are prolate or oblate ellipsoids suspended in a medium. He, too, has demonstrated that results calculated on the basis of his equation are in good agreement with experiment (40).

Substituting the data from Table III in Polson's equation

$$\frac{u}{u_0} = 1 + 4.0cV + 0.098cVk^2$$

where c = concentration of protein in grams per cc.

V = partial specific volume

k = axial ratio = $\frac{\text{length}}{\text{diameter}}$ of ellipsoid

gives a k value of 16.3 for molecularly dispersed gluten. Substitution of the same data in Simha's equation

$$\frac{u}{u_0} - 1 = cV\left[\frac{k^2}{15\left(ln2k - \frac{3}{2}\right)} + \frac{k^2}{5\left(ln2k - \frac{1}{2}\right)} + \frac{14}{15}\right]$$

leads to a k value of 17.1. Thus the results using the two methods are in essentially good agreement. Taking the mean of the two estimates gives a mean axial ratio of 16.7 for the molecularly dispersed gluten particle.

While the good agreement of the results using these two equations is reassuring it must not be overstressed. It is to be expected that they would tend to the same value since both are based on similar assumptions; that is, on complete Brownian movement among rotational ellipsoids. As has been pointed out by Robinson (34) it is of paramount importance that the basis of these equations be realized. If complete Brownian movement does not exist and the particles are to some extent oriented, viscosity is reduced and therefore the apparent value of k is lowered. The tendency towards orientation is particularly strong in capillary viscosimeters with their high rates of shear. Moreover, orientation is much more probable with greatly anisometric particles. Therefore, the value 16.7 is to be regarded as a lower limit until it can be confirmed by improved methods.

In spite of these limitations, if Perrin's formula (32) for prolate ellipsoids is used to calculate the $\frac{f}{f_0}$ corresponding to a k value of 16.7, we obtain a frictional coefficient of 1.86, which is in satisfactory agreement with McCalla and Gralén's figure of 1.92 after the effects of possible hydration are considered.

Molecular Volume and Radius

A combination of diffusion date and frictional coefficient permits an independent calculation of the molecular volume (1). Using the equation

$$\frac{f}{f_0} = \frac{kT}{D6\,\pi\,n\,V^{\frac{1}{3}}}\left(\frac{4\,\pi}{3}\right)^{\frac{1}{3}}$$

where k = Boltzmann's constant

n = viscosity of solution

V = molecular volume

D = diffusion constant

and the constants listed, we obtain a mean molecular volume for the gluten particle of 56×10^3Å^3. Calculation of the molecular volume of dry gluten from the density data listed also gives a value of 56×10^3Å^3. This agreement must be regarded as partly fortuitous, however, since the gluten molecule is almost certainly hydrated to some extent. Nevertheless, the close approximation indicates strongly that the hydration of gluten cannot be great or it would be reflected in a much larger molecular volume as calculated from diffusion data.

Combining the data for molecular volume from diffusion and the estimate of the axial ratio of the gluten particle from viscosity yields a radius of 9.3Å for the gluten molecule. For the reasons pointed out above, this figure is certainly a little low since the effects of hydration would tend to increase the radius. It is possible to get a second, independent estimate of the molecular radius from electrophoretic results. Abramson, Moyer, and Gorin (1, p. 128) give the equation for electrophoretic mobility of a randomly oriented long rod as

$$v = \frac{\sigma}{\eta}\left(\frac{1}{\kappa} + r_i\right)\chi$$

where ν = electrophoretic mobility

σ = charge density of particle

η = viscosity of solution

κ = Debye–Huckel parameter

r_i = average radius of ions in the ion atmosphere around the particle

χ is a shape factor, which, for long rods, has the form

$$\chi = \left[\frac{\dfrac{\kappa a}{(\kappa a + \kappa r_i)}\dfrac{K_0(\kappa a + \kappa r_i)}{K_1(\kappa a + \kappa r_i)} + \kappa a\, ln\left(\dfrac{a + r_i}{a}\right)}{1 + \kappa r_i}\right]\left[\frac{4}{F}\right]$$

where a = radius of rod

K_0 and K_1 are Bessel functions

and F is a function of κa, κr_i.

If the assumption is made that a prolate ellipsoid of great asymmetry approximates a long rod, this equation yields a molecular radius of 14Å. In contrast to the diffusion data this value must certainly be too high since it corresponds to a molecular volume of several times that calculated from density data. Such a degree of hydration is extremely improbable for reasons outlined before. Moreover, the relatively good agreement between the observed value of $\frac{f}{f_0}$ and that calculated from the axial ratio is direct evidence against such a large hydration. The discrepancy is certainly due to the rather wide limits of error in the original data and to deviations from the original assumptions of the Debye–Huckel theory of solutions upon which the equation is based. Solutions of 0.5μ ionic strength, even of uni-univalent electrolytes, are entering the region where no known laws are obeyed and therefore it is to be expected that the results will be approximate only.

Summarizing then, the mean radius of molecularly dispersed gluten must be somewhere between 10Å and 13Å. With the present data, of a low order of accuracy, it is not possible to set closer limits.

Discussion

These results serve to confirm and extend the concept of gluten as described by McCalla and Gralén (26). On the basis of ultracentrifuge and diffusion results they suggested that gluten was a reversible complex made up of molecules that varied progressively and continuously in both chemical and physical properties. On the observation of a high frictional ratio, the suggestion was made that these molecules were greatly elongated in shape but no data were presented to enable distinction between the possible alternatives of extremely high hydration of spheres or of a flattened shape. The enormous degree of hydration necessary to explain the diffusion values given in this study on the assumption of a spherical model, clearly exclude this possibility. Moreover, Simha, by his equation applied to viscosity data, has shown that the oblate ellipsoid model is extremely unlikely for gliadin (41). Since gliadin is a derivative of gluten, it is justifiable to extend his findings to the whole complex and so the prolate ellipsoid remains as the most likely model for the gluten molecule. In sodium salicylate solution, these ellipsoids of revolution are capable of existing singly or in aggregation, depending on the concentration of the salt in the solution and the protein fraction involved. If they are aggregated, the available evidence indicates that it is side by side association (26) rather than end to end attachment as in some viruses (38). The data given here indicate that the mean diameter of the particles making up the most soluble fraction of gluten is of the order of 25Å and their length of the order of 400Å to 450Å. This applies only to the molecularly dispersed fraction, of course. Very much larger aggregates undoubtedly exist in the ordinary dispersion. Again it must be emphasized that these are mean values, as the molecules, even in the molecularly dispersed fraction of gluten, are not homogeneous in mass.

It is interesting to compare the dimensions of the gluten molecule given above with those calculated for gliadin ten years ago by Neurath (29). Using Svedberg's estimates of 1.6 and 26,000 for the frictional coefficient and the molecular weight, respectively, of gliadin, Neurath found an axial ratio of 11.1 and a diameter of 18Å for the molecule of this derivative of gluten.

The mean surface charge density of gluten in sodium salicylate has a large, uniform, negative value that may be due to the reversible selective adsorption of the salicylate ions by the gluten. If this suggestion is valid it may offer a clue to the mechanism of dispersal of gluten in sodium salicylate. The bonds between salicylate ions and gluten particles may replace those between adjacent micelles, leading to the disintegration of the gluten mass. The sol so produced would then be stabilized by the high negative charge of the particles. Unfortunately, these data give no new information on the nature of the forces between gluten molecules or gluten aggregates. It was hoped that this method might suggest an explanation of the unique properties of gluten but this hope was unfulfilled.

In addition, these data give little new information on the extent of hydration of the gluten molecule. The observations recorded here are not of sufficient precision to permit a reliable calculation of the percentage hydration. Also, it seems doubtful whether the theory is sufficiently developed or the assumptions on which it is based sufficiently well fulfilled to justify the attempt at greater accuracy. In the writers' opinion, since the development of the lyophilization technique as applied to gluten removes many methodological barriers, a much more fruitful approach to the problem could be made by studying volume contraction. This has been used quite successfully by Neurath and Bull (31) on egg albumin.

References

1. ABRAMSON, H. A., MOYER, L. S., and GORIN, M. H. Electrophoresis of proteins. Reinhold Publishing Corporation, New York. 1942.
2. ADAIR, G. S. and ADAIR, M. E. Biochem. J. 28 : 199-221. 1934.
3. ADAIR, G. S. and ADAIR, M. E. Trans. Faraday Soc. 36 : 23-32. 1940.
4. ANSON, M. L. Advances in protein chemistry. *Edited by* M. L. Anson and J. Edsall. Vol. 2, pp. 361-386. Academic Press, Inc., New York. 1945.
5. BLOCK, R. J. Advances in protein chemistry. *Edited by* M. L. Anson and J. Edsall. Vol. 2, pp. 119-134. Academic Press, Inc., New York. 1945.
6. CLARK, W. M. The determination of hydrogen ions. The Williams & Wilkins Company, Baltimore. 1925.
7. DILL, D. B. and ALSBERG, C. L. Cereal Chem. 1 : 222-246. 1924.
8. FINDLAY, A. Practical physical chemistry. Longmans, Green and Co., London. 1938.
9. GETMAN, F. H. and DANIELS, F. Outlines of physical chemistry. John Wiley & Sons, Inc., New York. 1943.
10. GLASSTONE, S. An introduction to electrochemistry. D. Van Nostrand Company, New York. 1942.
11. HANDBOOK OF CHEMISTRY AND PHYSICS. Chemical Rubber Publishing Co., Cleveland. 1943.
12. JONES, D. B. Cereal Chem. 3 : 194-198. 1926.
13. KEKWICK, R. A. and CANNAN, R. K. Biochem. J. 30 : 227-234. 1926.
14. KEMP, I. Trans. Faraday Soc. 32 : 837-843. 1936.

15. KEMP, I. and RIDEAL, E. K. Proc. Roy. Soc. (London), 147A : 1-24. 1934.
16. KUHN, W. Kolloid-Z. 62 : 269-285. 1933.
17. LAUFFER, M. A. J. Am. Chem. Soc. 66 : 1195-1201. 1944.
18. LEY, H. and DIEKMANN, H. Z. physik. Chem. 106 : 161-177. 1923.
19. LONGSWORTH, L. G. Chem. Revs. 30 : 323-340. 1942.
20. LONGSWORTH, L. G. J. Am. Chem. Soc. 65 : 1755-1765. 1943.
21. LONGSWORTH, L. G. Ind. Eng. Chem., Anal. Ed. 18 : 219-229. 1946.
22. LONGSWORTH, L. G. J. Phys. & Colloid Chem. 51 : 171-183. 1947.
23. LONGSWORTH, L. G., CANNAN, R. K., and MACINNES, D. A. J. Am. Chem. Soc. 62 : 2580-2590. 1940.
24. LONGSWORTH, L. G. and MACINNES, D. A. Chem. Revs. 24 : 271-287. 1939.
25. LONGSWORTH, L. G. and MACINNES, D. A. J. Am. Chem. Soc. 62 : 705-711. 1940.
26. MCCALLA, A. G. and GRALÉN, N. Can. J. Research, C, 20 : 130-159. 1942.
27. MCCALLA, A. G. and ROSE, R. C. Can. J. Research, 12 : 346-356. 1935.
28. MACINNES, D. A. The principles of electrochemistry. Reinhold Publishing Corporation, New York. 1939.
29. NEURATH, H. J. Am. Chem. Soc. 61 : 1841-1844. 1939.
30. NEURATH, H. Chem. Revs. 30 : 357-394. 1942.
31. NEURATH, H. and BULL, H. B. J. Biol. Chem. 115 : 519-528. 1936.
32. PERRIN, F. J. phys. radium, 7 : 1-11. 1936.
33. POLSON, A. Kolloid-Z. 88 : 51-61. 1939.
34. ROBINSON, J. R. Nature, 143 : 923-926. 1939.
35. ROSE, R. C. and COOK, W. H. Can. J. Research, 12 : 63-81. 1935.
36. ROTHEN, A. J. Gen. Physiol. 24 : 203-211. 1940.
37. SCALLET, B. L. J. Am. Chem. Soc. 69 : 1602-1608. 1947.
38. SCHACHMAN, H. K. J. Am. Chem. Soc. 69 : 1841-1846. 1947.
39. SCHWERT, G. W., PUTNAM, F. W., and BRIGGS, D. R. Arch. Biochem. 4 : 371-387. 1944.
40. SIMHA, R. J. Phys. Chem. 44 : 25-34. 1940.
41. SIMHA, R. J. Applied Phys. 13 : 147-153. 1942.
42. SØRENSEN, S. P. L. Compt. rend. trav. lab. Carlsberg, Sér. Chim. 18 (5) : 1-124. 1930.
43. SOOKNE, A. M. and HARRIS, M. J. J. Research Nat. Bur. Standard, 23 : 299-308. 1939.
44. SPENCER, E. Y. and MCCALLA, A. G. Can. J. Research, C, 16 : 483-496. 1938.
45. SVEDBERG, T. and PEDERSEN, K. O. The ultracentrifuge. Oxford at the Clarendon Press, Oxford. 1940.
46. SVENSSON, H. Arkiv Kemi, Mineral. Geol. 22A (10) : 1-156. 1946.
47. TISELIUS, A. Nova Acta Regiae Soc. Sci. Upsaliensis, 7 (4) : 1-107. 1930.
48. WILLARD, H. H. and FURMAN, N. H. Elementary quantitative analysis. D. Van Nostrand Company, Inc., New York. 1940.
49. WOOD, T. B. and HARDY, W. B. Proc. Roy. Soc. (London), B, 81 : 38-43. 1909.

PHYSIOLOGICAL AND BIOCHEMICAL STUDIES IN PLANT METABOLISM

I. THE RESPIRATION OF THE SEEDLING WHEAT LEAF IN STARVATION AND ONTOGENY[1]

By George H. Duff[2] and Dorothy F. Forward[3]

Abstract

Uniform conditions for the culture of the plants and for conducting starvation–respiration experiments upon the first seedling leaves permit the recapture, and hence thorough investigation, of transitory physiological states. A standard sample of isolated, mature leaves, so produced and starved, is shown to be heterogeneous when tested by the tempo at which the tissues in different parts of the sample pass through the color changes that accompany starvation. These are correlated with respiration. Interleaf variation in tempo is relatively small. Intraleaf variation is maximal and the leaf is polarized at isolation but a complex redistribution of tempo within each leaf brings about depolarization and diminishes intraleaf heterogeneity as starvation progresses. The respiration of such a starving sample follows a time course of characteristic pattern, all the prominent features of which have their homologues in the corresponding patterns of younger and older leaves. The homologous characters undergo gradual modification with ontogeny. The respiration of the unstarved isolated leaf is very high and falls rapidly during growth but is low and declines slowly in maturity and senescence. At the transition from growth to maturity a slight, temporary rise in respiration occurs.

Introduction

In a wide area of the field of plant metabolism our knowledge is fragmentary because it has developed from studies of metabolic systems prominent in types of plants that are biologically remote from one another. The starch metabolism of the Solanaceae, the sugar metabolism of Monocotyledons, and the acid metabolism of succulent plants are familiar examples. Manifestly data have accumulated in this discrete manner for good reason and will therefore continue to do so. Nevertheless the circumstances invite attention to the advantage of a different approach, which is that of concentrating upon a single appropriate subject material an inquiry into as many aspects of its metabolism as possible. A series of researches instituted from this point of view has been and still is in progress in this laboratory. The results will be recorded in the present series of papers.

The experimental subject is the first foliage leaf of the seedling wheat plant, grown under uniform artificial conditions. It cannot be comprehended unless we take account of the succession of developmental stages through which it passes. From practical necessity we must also consider certain concurrently developing tissue and organ systems, but the main emphasis will be upon the leaf itself. It is typical enough of its kind and presents no marked peculiarities. It is exceptionally convenient in form and size and is adapted

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to the demands of the laboratory. An especially favorable property of the wheats is that they are readily cultured under artificial conditions (10). By so growing them the disturbing effect upon function of wide, unsystematic fluctuations in the conditions of growth can be minimized to the point of a rough standardization. For our purpose it is important to be able to return at will to a given physiological state or succession of states in order to examine separately as many aspects of these states as possible, yet always with the assurance that the data represent correlative attributes of the same state. Under favorable circumstances this advantage can be obtained for certain quantitative data within suitably narrow limits.

It is advisable to begin with the respiratory gaseous exchange because it is the most generally useful index of metabolic activity in the physiological organism. The experimental investigation of respiration in green tissues entails usually their isolation from the plant and always the imposition of darkness. If the observations are sufficiently long continued the tissues starve. In these circumstances, respiration rate, observed under uniform external conditions, does not remain steady but changes systematically in such a manner as to trace out in time a sequential pattern that is characteristic of the subject (2, 3, 4, 5, 6, 9, 11). The present subject is no exception to this rule. These patterned changes in rate are only in part determined by the controllable conditions of the respiration experiment itself. The determinants equally include the obscure internal factors that are inherent in the particular constitution which the interacting controls of development have produced. Some factors exert secular, others periodic effects upon the progressively changing respiration rate, thus characteristically influencing the pattern.

We look upon this pattern as a partial or guarded statement of the organism's metabolic constitution that it is our business to elucidate. Accordingly one of our objects will be to discriminate the factors determining the pattern, characterize their effects, and investigate the mechanism of their action. A contribution toward this end has already been made by Krotkov (8). Inquiries of this type take the physiological organism as the point of departure and proceed by the analytical consideration of such data as apply. On the other hand, some of our investigations begin at the other end of the organic scale with the distinction and separation of the enzyme systems. The probability of these two oppositely directed but complementary approaches reaching the desired common ground would appear to be greater when both courses are followed with the same subject.

In the present paper we shall restrict ourselves to respiration rate measured almost exclusively as carbon dioxide emission. Two kinds of progression of such rates must be considered. One denotes the sequence of metabolic states through which the wheat leaf passes during its growth, maturity, and senescence—an essentially ontogenetic progression. The other is essentially a starvation progression, manifested when the isolated leaf is maintained in the dark until it disorganizes. It is convenient to delineate the

former from data for the latter progression and these are derived through starvation experiments performed on leaves at different stages of development. Accordingly the matter presented in this paper is assembled in four sections: (*a*) procedure; (*b*) the starvation progression of a sample of leaves isolated at the standard stage of maturity, with evidence of heterogeneity in the sample; (*c*) the effect of age upon the starvation progression; (*d*) the ontogenetic progression of respiration rate.

Culture and Treatment of the Experimental Materials

Seed and Planting

To the present we have worked chiefly though not exclusively with *Triticum compactum* Host. var. Little Club and *T. dicoccum* Schübler var. Khapli. The latter has lent itself to our purposes particularly well and has been employed more frequently than the former. A full description of the cultural treatment of these materials follows and will serve the purposes not only of this but also of the majority of presently forthcoming papers in this series.

The seeds are planted in a light soil, which we maintain as uniform as possible. After a standard mixing and screening the soil is dispensed in equal quantities into waxed tin cans provided with drainage. Forty seeds are set out in a standard pattern, covered with a measured quantity of finely screened soil firmly pressed down and overlaid by two circles of filter paper. The latter are removed when the coleoptiles appear. A measured volume of tap water brought to temperature is given and the containers are transferrred to growing chambers having a capacity of 96 such containers.

The following external conditions are normally maintained: Range of dry-bulb air temperature, 21.7° to 22.4° C. in time at the position of the thermograph; about the same variation in space when lights are on, less when dark; mean in time and space 22.2° C. The relative humidity is under dew-point temperature control and hence varies systematically with the rapid cycle of dry bulb temperature variations in time, the mean being close to 55% of saturation at 22.2° C. The light is derived from eight tungsten filament lamps per chamber each of 500 w. The light passes through a one-half inch water screen over glass and gives about 750 ft-c. at the soil surface. The light periodicity is 12 hr. alternate light and dark. Air is circulated through the chamber at 2500 c.f.m. and is wasted and replaced at the rate of 150 c.f.m. A measured daily ration of water is given to each container and this is augmented as growth develops and is diminished as sampling of the population proceeds.

Growth and Development

Thus treated, the first coleoptiles of Khapli appear in a little more than 48 hours. At the end of three days the modal shoots are about 25 mm. long, the first leaf is entirely within the coleoptile, rolled up and not green in any part. A day later the first leaf is emerging, the terminal quarter of the blade is flat

and green, the second quarter green but rolled, the third quarter rolled and very pale green, the basal quarter rolled and shading in color from pale yellow-green distally to a colorless base. The blades are now about half grown and are 6 to 6.5 cm. long. During the fifth day the leaves expand to about 9 cm. in length but are otherwise generally similar to four-day leaves. By the end of the sixth day the first leaves are approaching full size, the second leaf is just emerging from the sheath of the first leaf, and the ligule of the first is only one-quarter of the length of the coleoptile from the top of the latter. The upper half of the leaf blade is mature in appearance, the third quarter is slightly rolled proximally and the basal quarter is rolled but green for three-quarters of its length, the remainder being pale green but not yellow-green. By the end of the seventh day modal first leaves are externally full grown and completely expanded with a length of 12 to 13 cm. The eight-day stage is our standard for the transitional or "adolescent" state in which the dimensional growth process is complete but residual internal changes are presumably in progress. The transitional period concludes on the 12th day. During this interval the plant becomes wholly autotrophic in its carbon nutrition.

On the 13th day the leaf quits the complex transitional state and enters the state of maturity. Our standard of maturity is the leaf on the 13th day from planting. Following this the external aspect of the leaf does not alter appreciably for about 10 days but shortly thereafter senescent changes become increasingly manifest in the terminal region and develop steadily until the tips of the leaves are yellowed and withered.

On the ninth day the rapidly developing second leaves are cut back in order to protect the first leaves from shading and for the sake of accessibility. Shoot growth continues but is cut back on alternate days so that the foliage of the seedling is restricted virtually to a single fully developed leaf.

Judging by the simple tests of relative size, form, color, and texture of the leaves the unnatural conditions of the growing chamber permit an adequate expression of the genetic characteristics of the wheats as they are normally manifested in the field or greenhouse. Several species and varieties have been grown and, depending upon the variety, the first leaves are from 25% to 35% longer at maturity than those of sister plants grown in the greenhouse. The green color is brilliant but slightly less deep than under good greenhouse conditions. The first leaves of the varieties maintain their characteristic differences of form, color, texture, and growth habit in the artificial just as in the more nearly natural environment. When such plants were allowed to develop without cutting back and grew to fructification under photoperiodicities of 15–9, 12–12, and 9–15, they fruited under all three, but fructification was accelerated by the augmented and retarded by the diminished photoperiod. The effect of the 12–12 photoperiod was deemed suitable for the purpose of developing a standard.

Selection and Sampling

An unselected population of seedlings invariably contains retarded or abnormal individuals originating from broken or deteriorated seed which cannot be sorted out before planting if the seed carries hulls. Such individuals are clipped out at the surface of the soil as they appear. The remaining seedlings are not strictly homogeneous but represent an appreciable range of difference in size and in the tempo of development. Each population is therefore subjected to a selection designed to reduce the range of these variations. A series of measurements of length from tip to ligule is made on about 200 leaves taken at random on or immediately following the ninth day. The distribution of leaf length is charted and the modal length determined. Fig. 1 shows that the range of size is quite wide and the distribution normal. The entire population is then tested by the scale and only those individuals lying within 0.75 cm. on either side of the mode are normally retained. All others are clipped out of the containers without disturbing the root systems. With an average sample of unselected seed the yield of acceptable leaves is 20% to 25% of the number of seeds planted.

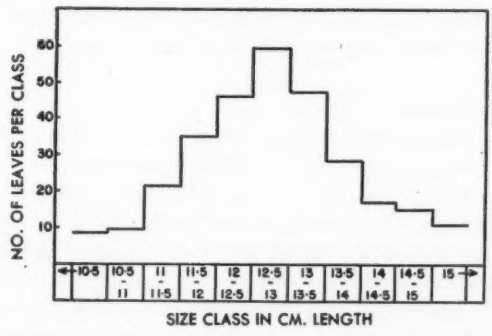

FIG. 1. *Distribution of leaf length in an unselected population of Khapli grown under standard conditions.*

The standard sample is composed of 50 leaves taken at random from the modal quartile of the population. At maturity in Khapli the sample weighs somewhat more than 5 gm. in the fresh condition and the leaves are 12 to 13 cm. long. The corresponding measures for Little Club are somewhat over 4 gm. in weight and 10 to 11 cm. in length. Such samples are convenient for most biochemical estimations as well as for our routine determinations of starvation respiration. Occasionally it has been advantageous to use larger samples as for instance, when the gaseous exchange must be measured over an unusually short interval of time or at very low temperature. Considerably smaller samples have necessarily been employed in studying the respiration of whole or divided leaves by micro-manometric methods. If close conformity to the standard conditions of growth has been successfully achieved the modal quartile will fall within the expected limits of size and

replicate samples of 50 modal leaves at maturity will yield routine respiratory data that agree to about 5%. Both elements of conformity are desirable in standard samples and normally both tests are realized. One or both may be disturbed by failure of control in the conditions of growth.

If the data are to be reproducible within sufficiently narrow limits, not only the conditions of growth but also the procedure for taking samples must be controlled. The starvation progression that we use as a general standard of comparison is that of the mature 13-day leaf. It is desirable that the general comparative standard should be a sample of relatively high sugar content and this we produce by giving 16 hr. of illumination instead of the normal 12 hr. on the day of sampling. The time at which sampling begins is determined by the number of samples to be taken simultaneously and is adjusted so that the manipulations are concluded shortly before the period of illumination ends. Handling the leaves as little and as gently as possible, they are cut at the ligule and stood in distilled water in groups of 50 in the growing chamber. When all the samples are complete they are rapidly weighed and returned to the chamber until the end of the 16-hr. period. They are then immediately removed to the laboratory and all are passed through the first steps of the further procedure within a very few minutes of zero time.

Respiration Determinations

The samples for respiration determination are normally placed in cylindrical glass respiration chambers containing a very little water immediately after weighing and thus stand in the growing chamber until zero time. The respiration chambers are then closed, covered with tin foil, and placed in the thermostat. An air stream of 1500 ml. per hour is freed of carbon dioxide by being passed through a very large tower of soda lime and then through a long Pettenkofer tube containing clear barium hydroxide solution so arranged as to offer very slight resistance to flow. This is started up immediately and whenever the initial rate is of critical importance a preliminary run of 15 min. is allowed for clearing the system of existing carbon dioxide before estimations begin. Except where temperature effects are under examination, the thermostat temperature is identical with the mean temperature of growth (22.2° C.) and is uniform to less than 0.1° C. The evolved carbon dioxide is estimated by the Pettenkofer method adapted to continuous operation by an automatic air current commutator, which switches the air stream from one absorption tube to the next of a closed series, usually every three hours. Estimations are continued without interruption until tissue disorganization is evident. This requires about 115 hr. for the 13-day leaf at 22.2° C. The leaves are examined daily without opening the respiration chamber or stopping the air stream and the externally visible changes are recorded. The respiration rates are calculated as mgm. carbon dioxide per 100 gm. of fresh tissue per hour. Other methods of determining respiration must be used for special purposes

but since few such data are to be considered in this paper, these methods will not be referred to here except to note that the results by all the methods are reconcilable.

The Starvation Progression at Maturity

Course of the Progression

Fig. 2, A gives the respiration records of duplicate samples of mature Marquis wheat seedling leaves from a population grown in the greenhouse in July and isolated at the close of a long summer evening. These records are

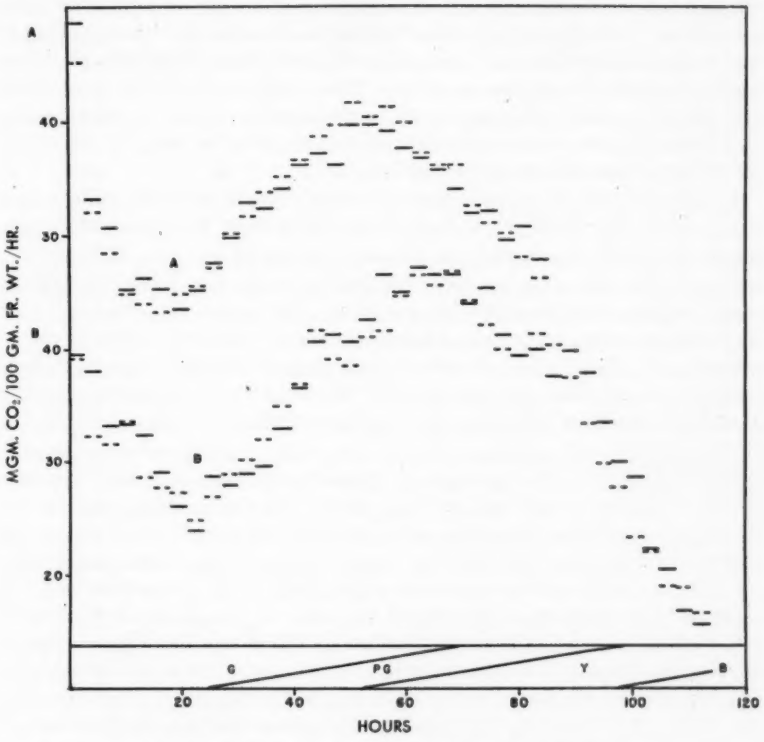

FIG. 2. *Starvation respiration of mature first leaves of wheat seedlings.*
A. Marquis grown in the greenhouse in July and isolated at dusk.
B. Khapli grown in plant chamber under standard conditions and isolated in full light. The diagram along the time axis indicates the changes of color that occur as starvation progresses. G—green; PG—pale green; Y—yellow; B—gray-brown.

presented first because they are devoid of certain complications that are present in the records of standard samples of Khapli and Little Club and that appear to be related to the sharp and exact photoperiodicity of the standard

conditions of growth. The complicating occurrences distort the form of the progression and though the undistorted form is deducible it is manifestly desirable to demonstrate it experimentally. Fig. 2, A presents the undistorted form as we observed it early in our work before any hypothesis of the origin and effects of the complicating factors had been developed.

The progression begins with a high but falling rate, which declines in a smooth down-valley curve to a transitory minimum. This is succeeded by a phase of almost uniformly accelerating respiration, which maximates and is followed by a phase of substantially uniform deceleration.

Fig. 2, B shows the course of the progression in duplicate standard samples of Khapli isolated on the 13th day from planting following 16 hr. of continuous illumination. The two samples were selected at random from amongst eight sister samples which had been taken simultaneously from the same population. Sample 1 happened to have the greatest fresh weight and Sample 2 the least but for one of the set of eight samples. The pair are a little less concordant in the early hours but rather more concordant in the later hours of starvation than the generality of well found duplicates.

The standard progression is evidently of the same three-phase form as that represented in Fig. 2, A, but in each of the three arms there here occur subsidiary inflections interrupting the smooth progression by either a pause or a temporary reversal of its sense. The first subsidiary inflection invariably occurs in all standard records, the second nearly always, but the third is in every respect more variable than the others and is frequently little or not at all developed. They are regarded as a superimposition upon the simple form of Fig. 2, A and, from the character of the distortion they produce in the simple form, the term "transposition" has been applied.

Accompanying the progression of respiration rates there proceeds a sequence of color changes in the leaf tissues that is similar to those described for other starving leaves and, like them, correlative to the respiration rate (6, 1). The original green color of the leaves has become perceptibly paler by hour 24 when the rising phase begins. The fading green passes into yellow that first appears more or less centrally in the leaf blades at about the 50th hour and progressively involves the whole blade except for very small amounts of tissue at the extremes, which tend to remain green. Later the color passes to gray-brown. The progress of these color changes is indicated in Fig. 2. This visible breakdown of chlorophyll denotes the concurrent progress of other deteriorative processes (6) which culminate in failure of semipermeability in the leaf cells and this results in the exudation of sap into the intercellular spaces. The injected appearance of the leaf blades that this event induces gives us a visible endpoint to the progression. Usually the respiration rate departs from the regular progression as this terminal stage supervenes and it may rise or fluctuate irregularly as autolysis rapidly involves more tissue. It is evident that at this point the physiological condition of the tissues has passed and the carbon dioxide evolved is no longer that of an organized respiratory metabolism. In the standard progression of Khapli leaves about 10% of the leaf

tissue becomes injected and respiration rate becomes irregular at or about hour 115 and these joint indications are taken to mark the end of the starvation progression.

The form of the progression evidently presents the same underlying characteristics as those of the mature leaves of durable physiological types that authors of the Cambridge school have considered (1, 2, 4, 6). The barley leaves which were the subject of Yemm's experiments (12, 13), though closer to our subject materials systematically, do not appear to show this form. We are therefore investigating a somewhat differently organized subject. The distinction from the durable types is prominently manifested in the time scale, which in the cherry laurel leaf is several times that of the wheat leaf and the denoted sequence of metabolic states is correspondingly well spread out in the one and foreshortened in the other. In the wheat leaf the progression covers a period of five days only and the successive somewhat overlapping phases pass by quick transitions from one to the next after a relatively brief duration. Though this fugitive quality imposes certain experimental limitations, the very abruptness of the transitions effectively calibrates the time scale in physiological dimensions and thus assists in the correlation of independently determined metabolic data with those for respiration.

The effect upon the progression of the constitutional difference between the durable types and the wheat leaf is also conspicuous in the minimum phase to which the descending first arm of the progression leads in them all. In the durable types the minimum may be much extended (2) and clearly denotes a relatively steady metabolic state that is distinct from preceding and succeeding states. In the wheat leaf the minimum seems by inspection manifestly transitional and this indication is confirmed in Krotkov's analysis of the progression (8) in terms of the ultimate substrate sources of the evolved carbon dioxide. Two components are recognized. One is identified with the first declining phase of the progression and its kinetics are held to be those of the metabolism of the sugar reserve. The other corresponds to the rise and fall of respiration that succeeds the minimum. The kinetics are complex and are dominated by the entry into down-grade metabolism of structural substances of the tissues of which the chloroplastic components stand merely as a type. These degenerative processes begin before the depletion of sugar is complete and, if these are the only components of the system, the overlap of the two changing rates, the one progressively diminishing and the other augmenting, must bring about the transitional minimum at the time when the two accelerations of opposite sign become numerically equal. In considering the two-component hypothesis we shall refer to the components as the depletion and the degeneration component respectively. Similar terms will be applied to the corresponding phases of the progression.

It is obvious that in so far as the time courses of the starvation progression of standard samples at the mature stage are congruent, the total carbon dioxide produced in the course of starvation must be uniform. The total carbon dioxide production by seven standard samples determined by ourselves

on Khapli 681 of three successive seed years, incidentally to work on the chemical constituents and not for the purpose of examining this datum in particular, ranged from 3928 to 4199 mgm. carbon dioxide per 100 gm. of leaves with a mean value of 4058 mgm. But though variations of limited range in the temperature of starvation exert a marked effect upon the time course of the progression, certain characteristics are not determinably influenced and amongst them the integrated carbon dioxide of starvation. Uniformity of carbon dioxide production therefore rests upon more than coincidence of rate along the time scale. An apparently similar relation has been observed in fruits (7). On the other hand the integrated carbon dioxide is strongly influenced by varying such conditions as lead to variation in the content of respiratory substrate at the time of isolation. The circumstances suggest a relation between substrate content and integrated carbon dioxide that will be considered in future papers.

Physiological Heterogeneity of the Standard Sample

Before the conclusions from data for the standard sample can be formulated in terms of cellular function, we must take into account the physiological heterogeneity of the sample and consider both intraleaf and interleaf variations. The latter are the less problematical partly because they can be minimized through the method of culture, selection, and sampling. On the other hand there is no reason to believe, a priori, that the intraleaf variation can be correspondingly minimized by these means. An experimental inquiry into the magnitude and physiological controls of intraleaf heterogeneity is therefore inescapable and is begun though by no means concluded here.

Since the data at present under consideration are for sequences of metabolic state the element of heterogeneity that first attracts attention lies in the difference of speed at which the progression is passed through. This element we denote as "tempo". The standard sample displays both interleaf and intraleaf heterogeneity in tempo. Observations are readily made because, as noted in the preceding section, the progressive changes in respiration and metabolic state during starvation are accompanied by correlative changes in superficial color that can be observed in individual leaves and in the various parts of a single leaf. It is obvious that allowance must be made for a certain disparity arising from the fact that the observed color changes apply to the chlorenchyma tissues alone, whereas the data for respiration apply to other cell types as well though the latter are in small minority.

Our routine observations of the progressive color changes that occur during starvation show that the vast majority of the leaves undergo these changes so closely together that they cannot be distinguished as to tempo by this means. They also indicate that in the standard sample the intraleaf variation in tempo exceeds the interleaf variation sufficiently to appear as the definitive statistical element of the total heterogeneity. To define the heterogeneity more clearly we evaluated daily the color grade of each quarter of every leaf of a starving standard sample by matching the colors visually under uniform

conditions to a series of standards. These observations showed that the initial color grade of the unstarved sample was substantially uniform. After 24 hr. of starvation at 22.2° C., however, the sample by this test showed itself determinably heterogeneous in tempo, differences appearing both as between leaves and within the leaf.

Regarding first the interleaf variation, it was found that at the 24th hour about 85% of the leaves were indistinguishable as whole leaves. The variant 15% were not randomly distributed but were all laggards of slightly lower tempo than the large, modal group. This distribution cannot be assumed to persist throughout starvation but is probably complicated by changes entailing some increase in interleaf heterogeneity as starvation proceeds. But there is reason to believe that it applies to the first 36 hr. or more and our most critical data derive from this interval.

Taking the colors of the four quarters of the leaf separately as an index to the intraleaf heterogeneity at that time, the distribution of color grade at the 24th hour is shown in Fig. 3. It is evident that at this point the basal

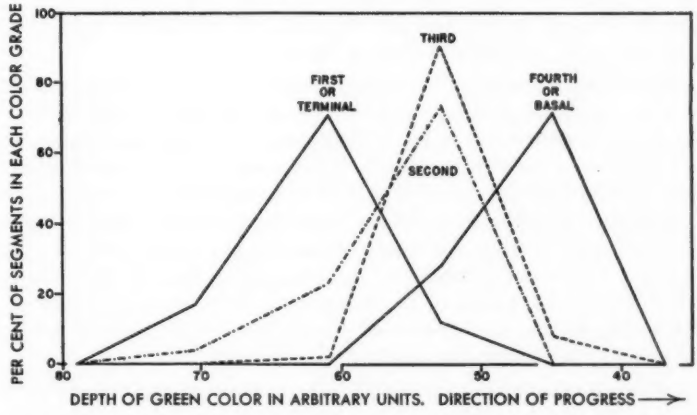

FIG. 3. *Distribution of color depth in the four quarters (from tip to base) of a standard sample of intact Khapli leaves at the 24th hour of starvation.*

region has the highest and the terminal region the lowest tempo. The two central quarters have identical modes but the distribution of the variants relates each of the central quarters to its neighbouring extremity so that a gradient exists though it is not uniform. This distribution of tempo does not persist but, as shown in the previous section, the central region overtakes the basal by the time yellowing commences at about hour 50. From this we must infer either that the earlier and later stages of chlorophyll degeneration are unrelated, which is improbable, or more probably, that tempo is redistributed as starvation progresses. Further light is shed on this question by the observations that follow.

When seedling plants are transferred to the dark and the first leaves starved without severance, a sequence of color changes occurs which is comparable to that of the isolated leaf except that it develops in a marked gradient and, at the same temperature, takes considerably longer to conclude. The terminal region is the first to yellow, the basal region is the last, and a gradient connects them. Evidently the attached leaf is characterized by a tip to base polarity in tempo which is maintained through the yellowing stages of starvation. As we shall show below, this is also the initial condition of the isolated leaf. Yet 24 hours of starvation after severance effects a complete reversal of the initial polarity and brings about the condition represented in Fig. 3. Then in the course of the next 24 hours the distribution changes further, bringing the central region instead of the basal into the most advanced position by the time yellowing begins. In all probability this complex redistribution proceeds continuously and apparently with a progressively diminishing amplitude of swing. It seems clear that its main statistical effect is twofold: In the first place it ultimately replaces a highly systematized or polarized distribution of tempo with a more nearly random or depolarized distribution. In the second place, the range of heterogeneity is diminished inasmuch as the time taken for the leaf to pass through the stages of chlorophyll degradation is considerably less in the isolated than in the attached leaf.

The factors in control of the initial polarity and those underlying the redistribution will be examined in future papers. In one of these it will be shown that when freshly isolated leaves are immediately cut transversely into quarters which are then starved concurrently but separately in a manner closely similar to that in which the standard sample is starved the terminal quarters strongly tend to turn yellow first and the basal quarters last with the central quarters intermediate. But the quarters themselves yellow first in their central parts. These effects of quartering demonstrate that the initial distribution of tempo in the leaves of an isolated standard sample is that of a gradient from tip to base and show that organic unity is a condition both of the initial polarity and of the subsequent depolarization.

Thus, judging by the limited physiological test of tempo, the chlorenchyma cell population of the attached leaf is marshalled in a polarized series and a maximal range of heterogeneity in tempo is maintained. We may suggest that this denotes a fully organized condition of the cell population and is a correlative of its role as a part of the intact organism. Upon severance such elements of organization as are contingent upon organic unity with the plant must necessarily be abated. What we observe under these circumstances is an abatement of the original polarity and of the original high degree of heterogeneity in the tempo of the starvation color changes. The characteristics of greater intraleaf homogeneity that develop as starvation progresses to the yellowing stage may thus be regarded as the insignia of a progressively lowering grade of organization. At the simplest, both the intercellular and intracellular aspects of organization will have to be considered. The former is evidently abated by isolation though presumably other factors are also influential.

The abatement of the latter is induced through starvation. It seems clear that the redistribution of heterogeneity in tempo must influence the sequence of metabolic events during starvation of the standard sample and thus cannot be disregarded in an analysis of the starvation progression.

There can be no doubt that the changes of color grade and respiration rate are correlative but the relation is clearest with the degeneration phase of the starvation progression. Observations have usually been made at the fairly advanced stage of yellowing and indicate that this stage is marked by increasing respiration rate (1, 2, 6). The relation between tempo and respiration in the earlier stages of starvation is not correspondingly clear and the phenomena accompanying depolarization suggest that it may be far from simple. The data of Fig. 4 show that under appropriate conditions the respiration rate in the initial stages of starvation has a polarized distribution like that of tempo. The data were derived from a group of 12 mature Little Club leaves which were quartered soon after gathering and the oxygen uptake of

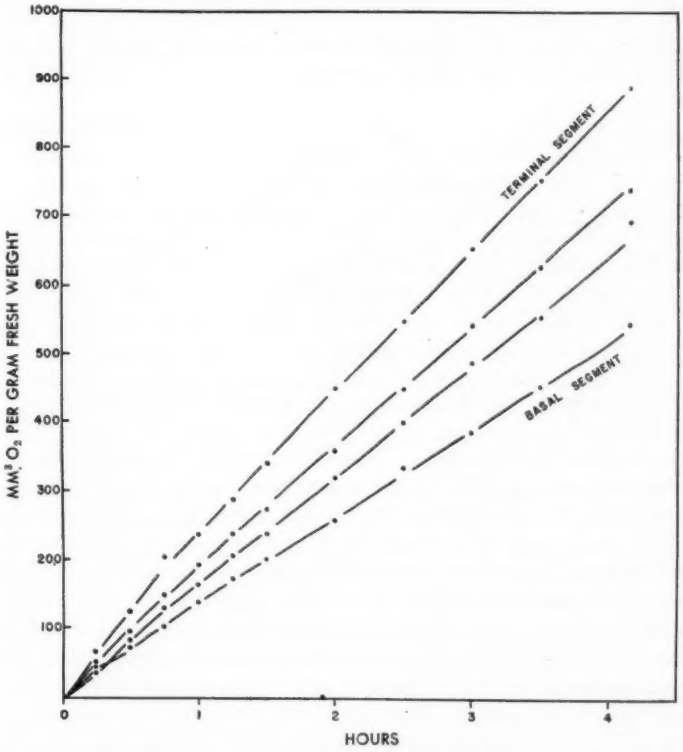

FIG. 4. *Time progress of oxygen uptake in the four separated quarters of standard, mature leaves of Little Club wheat, showing a gradient of respiration from terminal quarter to basal quarter.*

the composite four quarters determined manometrically. Calculated on the fresh weight basis a gradient in respiration is evident, but in a forthcoming paper a co-worker will show that the initial respiration gradient changes with ontogeny and is very susceptible to the influence of factors associated with modified water relations. The data of Fig. 4 therefore are not by themselves definitive. Nevertheless they demonstrate the reality and magnitude of the intraleaf heterogeneity in respiration rate.

Effect of Age upon the Starvation Progression

Fig. 5 gives three records of Little Club cultured under standard conditions from seed grown at Moscow, Idaho, and treated experimentally as described in an earlier section. The samples were isolated on the 6th (A), 13th (B),

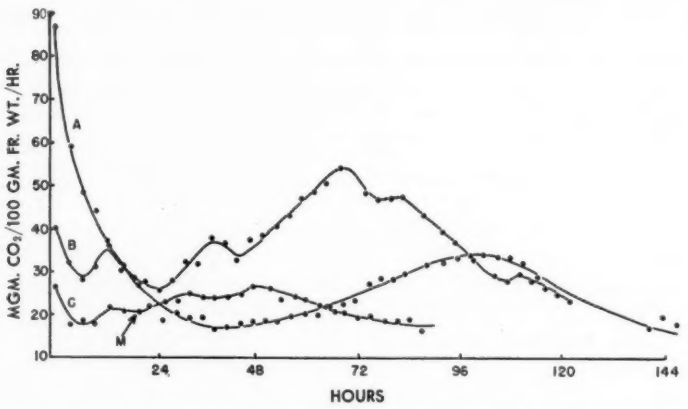

FIG. 5. *Starvation respiration of standard leaves of Little Club isolated at three stages of ontogeny: A (juvenile), 5 days; B (mature), 13 days; C (senile), 40 days from planting.*

and 40th (C) days from planting. Data for many intermediate stages have been derived but our immediate purposes can be served by the three examples. It is evident that the two extreme records are variants of the form typified by the standard of maturity (5B), which has already been considered. They must therefore present homologous characteristics, the comparison of which over the entire series would describe the effect of age upon the starvation progression. The more prominent of such characteristics are (*a*) the initial rate, (*b*) the duration of life and total carbon dioxide production, (*c*) the dimensions of the depletion phase and the minimum, (*d*) the dimensions of the degeneration phase, (*e*) the transpositions. Each of these manifestly undergoes modification with age. A thorough-going analysis must await the presentation of matter that cannot be recorded here, but the broad outlines of the changes occurring along the age scale will be formulated.

Initial Rate

This attribute is clearly very high in young, growing leaves and falls off with age. The data of Fig. 5 show that the decline is very rapid during the premature and is much more gradual in the postmature stages. It is clear that initial rate must be considered in connection with the ontogenetic progression of respiration rate and we therefore reserve the further data for the following section of this paper.

Duration of the Progression

The terminal condition of breakdown is reached in 6-day leaves in about 144 hr., in 13-day leaves in about 115 hr., and in 40-day leaves in about 87 hours. The duration of life in starvation thus contracts from youth through maturity to old age but most of this contraction occurs actually at the extremes of the age scale. The total production of carbon dioxide is manifestly much smaller in old age than at any earlier stage. The difference between the 6-day and 13-day leaf in this respect is in favor of the former but is much smaller than the difference between the 13-day and 40-day leaf. The prominent distinction between 6- and 13-day leaves lies in the distribution of the carbon dioxide production as between the depletion and degeneration phases.

Depletion Phase

The very high initial rate of the young leaf is associated with the occurrence of a depletion phase of corresponding dimensions which requires 40 hr. to reach the transitional minimum. The decline of respiration rate in this phase is characteristically smooth and unbroken by a transposition with the result that the simple form of progression (cf. Fig. 2,A) is manifested in the growing leaf. The total carbon dioxide production of the depletion phase in premature leaves is substantially greater than that of any subsequent stage.

The depletion phase of the mature leaf, beginning with its much lower initial rate, requires 24 hr. to reach the transitional minimum and a much smaller amount of carbon dioxide is produced than in the premature leaf in this phase. The phase is interrupted in the instance before us by a particularly prominent transposition which so divides the phase that the simple form is much distorted and obscured. This transposition is always present but not always so strongly developed as in Fig. 5, B.

In the 40-day leaf further contraction in the dimensions of the depletion phase is manifest. The pitch of respiration is low and the carbon dioxide production correspondingly small. The extent of the phase in time is obscured by the transpositional rise and by the fact that the post-transpositional decline to the minimum is in this case so slight that the minimum could not be confidently identified by inspection. Because of the transpositions it has required a very close comparative study of several full series to decide the homologies of the much reduced respiration records of senescent standard leaves. These considerations will be presented in a following paper the conclusions of which we must here anticipate to the extent of identifying the point marked *M* in

Fig. 5, C as the homologue of the more evident minima of Figs. 5,A and 5,B. This identification leads to the conclusion that the extent of the depletion phase in time is but slightly less in the senile than it is in the mature leaf.

Degeneration Phase

The onset of the degeneration phase, which begins with the transitional minimum, occurs progressively earlier during growth. During maturity and to a fairly advanced stage of senescence the degeneration phase is initiated under standard conditions with great regularity at the 24th hour from isolation and darkening of the leaves. This period becomes somewhat shortened as the leaves pass into the remote stages of senescence. The duration of the degenerative phase in 6-day leaves is somewhat over and in 13-day leaves is somewhat under 100 hr., but in the 40-day leaves the interval has declined to about 66 hr. The carbon dioxide production of the degenerative phase increases slightly in premature leaves with advance in age, the mature leaf having maximal production. During senescence the production declines at an increasing rate till in 40-day leaves it is about half that of mature leaves.

In all the leaves we have examined that were cultured and observed under standard conditions the degeneration phase is present and has the form of a rise to a maximum followed by decline. Sometimes in advanced stages the peak may be considerably flattened and the falling arm may then not decline to values as low as the minimum before the terminal disorganization supervenes. In the durable physiological type of leaf represented by the cherry laurel (6) the starvation progression in juvenile leaves is devoid of the rise and fall that characterizes the degeneration phase of mature leaves of this species and of wheat leaves of all ages. But the time scale of age in the wheat leaf is so short that the tissues of an isolated sample possibly make considerable progress ontogenetically during the course of a starvation experiment in which event observation of the penultimate stages of starvation with the tissues remaining in a juvenile state might be difficult.

Transpositions

The occurrence of these inflections in the respiration records of mature leaves has been referred to in the preceding section. Fig. 5 shows that they are not present in the first declining arm of the record of the 6-day Little Club leaf. The early transpositions appear as the leaves cease growth and undergo the transition to maturity. In Khapli they (or their equivalent) appear somewhat earlier, in the 7-day leaf. Apparently the wheats differ in this respect. The number of juvenile records we have observed is far fewer than the number of mature records but they support the statement that the early transpositions are absent (or exceedingly weakly developed) in the records of growing leaves but are invariably present in those of all mature and postmature leaves cultured under standard conditions.

The Ontogenetic Progression of Respiration Rate

The progression of respiration rate with age should ideally be delineated by some measure of respiration that has not been affected by depletion nor by any other variable factor except the age factor itself. The observed initial rate of the starvation progression complies substantially with the stipulation regarding depletion but not with the more general stipulation. It will be shown by a co-worker in a following paper that initial rate, as here derived, is in all probability affected by excitation of the leaves induced by the handling incidental to the preparation of the sample. Thus the initial rate is higher than that proper to the unexcited state. Also the excited initial state is not an adjusted or drifting steady state but is perturbed, and the perturbation lasts for several hours. These effects cannot be corrected for as yet, but in some instances they may be evaded by the adoption of another measure. It will be shown by methods adapted to following rapid changes in respiration rate that the perturbation passes with the beginning of the transposition and that the rate then settles down more or less briefly before the transpositional rise occurs. Though depletion is probably effective by this time, there is reason to believe that this could be allowed for. This adjusted transpositional rate might then be adopted uniformly as the measure of respiration for determining the ontogenetic progression were it not that the starvation progressions of young leaves lack the early transpositions. In the circumstances we present the ontogenetic progression over the whole age series in terms of initial rate and supplement these data with those for transpositional respiration rate in the mature and postmature stages where early transpositions invariably occur.

Numerical values of initial rate are given in Table I and plotted in Fig. 6 (A and B) for both wheats. Two populations of Khapli and one of Little Club provide the available *ad hoc* data. Supplementing these a great many observations of initial rate have been made incidentally, all of which confirm the essential features of the progression as here delineated. The populations were grown under the standard conditions described in Section 1 except that the dry bulb temperature varied beyond standard limits and the mean hourly temperature was a little below the standard. Also the plants were removed from the growing chambers to a dark room and back again each day. Since the frequent handling of the containers has a slight retarding effect upon development, the result of these two circumstances is to extend the time scale as here presented somewhat over that characteristic of strictly standard conditions. No other difference is observable.

Fig. 6 portrays the rapid decline of initial rate during the later growth stages of the leaf when stretching is the dominant phase of growth but not the only growth activity in progress. By the time dimensional growth has ceased (eight days, cf. Section 1) respiration is an order of magnitude lower and thereafter changes very much more slowly. The rates of the youngest observed leaves are so high and their decline is so steep that the question of the magnitude and course of respiration in the antecedent stages is raised. To shed

TABLE I

ONTOGENETIC PROGRESSION OF RESPIRATION RATE

Days from planting	Initial respiration, mgm. CO_2/100 gm./hr.			Transpositional respiration, mgm. CO_2/100 gm./hr.	
	Little Club 8	Khapli 7	Khapli 8	Little Club 8	Khapli 8
5	111		99.5		
7	44.3		56.9		
9	37.0	43.6	41.5		
12		48.9			
13	40.5		42.9	31.5	32.0
19	31.6		37.4	25.0	30.0
22		37.1			
23	31.8		38.0	25.5	33.0
27	29.1		32.6	22.5	26.0
28		36.5			
32	28.1		30.4	22.2	23.5
35		26.6			
36	31.5		27.7	21.5	19.5
40	26.5			19.5	

some light on this question seeds of Little Club were germinated in moist chambers at 22.2° C. and, when the stage characteristic of about 48 hours' growth under standard conditions (soil) had been reached, the embryos were

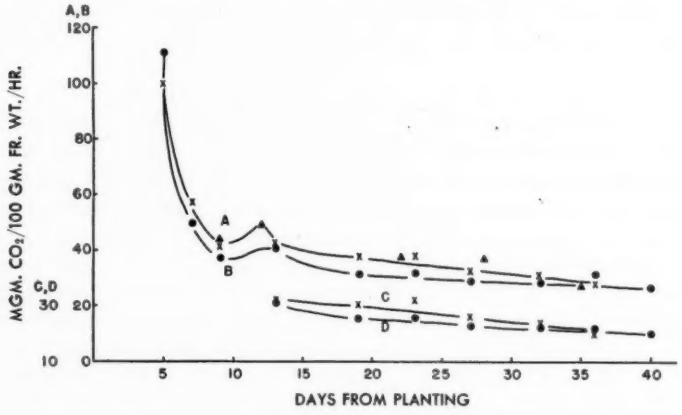

FIG. 6. *Respiration of standard samples of Khapli and Little Club leaves isolated at various times during growth, maturity, and senescence.*

A. Initial (unstarved) respiration of Khapli; crosses and triangles indicate the values derived from two populations grown on different occasions.

B. Initial (unstarved) respiration of Little Club derived from a population grown concurrently with one of Khapli.

C. Transpositional respiration of Khapli.

D. Transpositional respiration of Little Club.

Values plotted for C and D were both derived from the concurrently grown populations of A and B.

separated from the endosperms and their respiration separately determined at 22.2° C. after the manner of Barnell's experiments with barley (3). The initial rate proved to be of the order of 300 mgm. carbon dioxide per 100 gm. fresh germlings per hour. The exceedingly rapid decline in rate per unit mass of fresh tissue therefore probably continues for some time previous to the 5th day. Since stretching is in progress throughout this time in one part or another of the heterogeneous tissue systems, the enormous uptake of water that characterizes the stretching phase must account for a great part of the sharp decline in rate of carbon dioxide production per unit mass of fresh tissue. Since germination metabolism begins with low rates it is evident that the sharp decline in rate here observed must be preceded in a given tissue by an even sharper rise and at some early stage a maximum must be developed.

Between the 8th and 12th days the seedlings make the transition to a completely autotrophic carbon nutrition. Though dimensional growth is complete by the 9th day it is probable that some processes of structural differentiation are continuing in the leaf for the next day or two. During this interval also the vertical posture of the seedling leaf gives way to a lateral posture which must entail considerable internal differences arising from the incidence of radiation. The physiological state of the tissues is thus in all probability a complex one as the characteristics of juvenility fade out and are replaced by those of maturity. The data of Fig. 6 indicate that a slight, transitory rise in initial rate occurs during this interval. We were at first uncertain of the reality of this rise but confirmatory evidence of its reality in the case of Khapli will later be presented by co-workers. Accordingly, we incorporate the transitory rise as a formal characteristic of the ontogenetic progression when the progression is delineated in terms of initial rate.

These complications are normally past by the 13th day and because this is the earliest time of which this is true we have adopted the 13-day leaf as our standard of maturity. Owing to the slight departures of growth conditions from the narrow range of the standard, the 13-day leaves of the present series appear to be not quite clear of the transitory rise. Following the 13th day, however, it is evident that the initial rate gradually declines throughout maturity and into senescence. The values give evidence of increased variability after about the 30th day and this we have treated as a random scatter which presumably indicates an increasing heterogeneity of the population as progressive disorganization becomes important (cf. Section 1). In this connection the regularity of the data in the earlier stages may be remarked. This is especially manifest in the case of Khapli for which the data of two populations grown at different times from the same seed material are available. One population was grown and experimented upon concurrently with that of Little Club and the two sets of data therefore apply to strictly parallel material. The other population of Khapli was independently grown yet shows the same differences from Little Club. It is clear that while the ontogenetic progression is closely similar in the two wheats, a consistent, if

narrow, margin of numerical difference is demonstrable. The three starvation progressions for Little Club given in Fig. 5 were taken from the series which yielded the initial rates given in Fig. 6, B.

We may conclude by surveying the ontogenetic progression as far as it can be given in terms of transpositional respiration rates. These are also recorded in Table I and Fig. 6 (C and D). Where the transposition entails a definite rise in rate the median value between the extremes is taken, otherwise the transpositional value itself. The transpositions in Little Club always entailed a rise but this was true of Khapli in the 13-, 19-, and 36-day records only. In the other three Khapli records the transposition took the form of a pause in the decline of rate. As far as the data go it is clear that though the numerical values are lower than those for initial rate the progress in time is substantially the same. A possible exception to this is the slight rise that is suggested at about the 23rd day when the leaves begin to give external evidence of senescent changes (cf. Section 1). Fig. 6 shows that an overliteral interpretation of the numerical values for initial rate would lead to the same conclusion. With transpositional as with initial rates the two wheats maintain a similar, narrow margin of difference from one another. We conclude that as far as they can both be applied, the two respiratory measures give substantially the same account of the ontogenetic progression.

Acknowledgments

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References

1. AUDUS, L. J. Mechanical stimulation and respiration rate in the cherry laurel. New Phytologist, 34 : 386-402. 1935.
2. AUDUS, L. J. Mechanical stimulation and respiration rate in the green leaf. II. Investigations on a number of angiospermic species. New Phytologist, 38 : 284-288. 1939.
3. BARNELL, H. R. Analytic studies in plant respiration. VII. Aerobic respiration in barley seedlings and its relation to growth and carbohydrate supply. Proc. Roy. Soc. (London), B, 123 : 321-342. 1937.
4. BLACKMAN, F. F. The manifestation of the principles of chemical mechanics in the living plant. Rept. Brit. Assoc. Advancement Sci. 884-901. 1908.
5. BLACKMAN, F. F. and PARIJA, P. Analytic studies in plant respiration. I. The respiration of a population of senescent, ripening apples. Proc. Roy. Soc. (London), B, 103 : 412-445. 1928.
6. GODWIN, H. and BISHOP, L. R. The behaviour of the cyanogenetic glucosides of the cherry laurel leaf during starvation. New Phytologist, 26 : 295-315. 1927.
7. KIDD, F. and WEST, C. Physiology of fruit. I. Changes in the respiratory activity of apples during their senescence at different temperatures. Proc. Roy. Soc. (London), B, 106 : 93-109. 1930.

8. KROTKOV, G. Carbohydrate and respiratory metabolism in the starving, isolated leaf of wheat. Plant Physiol. 14 : 203-226. 1939.

9. KROTKOV, G. Respiratory metabolism of McIntosh apples during ontogeny. Plant Physiol. 16 : 799-812. 1941.

10. VAN DE SANDE-BAKHUYZEN, H. L. Studies in wheat grown under constant conditions. A monograph on growth. Stanford Univ. Food Research Inst. 1937.

11. WALFORD, E. J. M. Studies of the tomato in relation to its storage. I. A survey of the effect of maturity and season upon respiration of greenhouse fruits at 12.5° C. Can. J. Research, C, 16 : 65-83. 1938.

12. YEMM, E. W. Respiration of barley plants. II. Carbohydrate concentration and carbon dioxide production in starving leaves. Proc. Roy. Soc. (London), B, 117 : 504-525. 1935.

13. YEMM, E. W. Respiration of barley plants. III. Protein catabolism in starving leaves. Proc. Roy. Soc. (London), B, 123 : 243-273. 1937.

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